

FINAL REPORT

Evaluating the Importance of Precursor Transport and Transformation for Groundwater Contamination with PFAS

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ABBREVIATIONS AND ACRONYMS

AFCEC: Air Force Civil Engineering Center
AFFF: Aqueous Film Forming Foam
AMPA: Aminomethyl phosphonic acid
DGT: Diffusive gradient in thin film
DOC: Dissolved organic carbon
ECF: Electrochemical fluorination
EOF: Extractable organofluorine
ESI: Electrospray Ionization
FASA: Perfluoroalkyl sulfonamides
FBSA: Perfluorobutane sulfonamide
FHxSA: Perfluorohexane sulfonamide
FOSA: Perfluorooctane sulfonamide
FT: Fluorotelomerization
FTSA: Fluorotelomer sulfonates
HLB: Hydrophilic lipophilic balance (HLB) sorbent
JBCC: Joint Base Cape Cod
 K_{ai} : Air-water interface partition coefficient
 K_d : Sediment-water partition coefficient
 K_{mw} : Membrane-water partition coefficient
 K_{sw} : Sorbent-water partition coefficient
MCMC: Markov Chain Monte Carlo simulations
NIST: National Institute of Standards and Technology
OD: Optical density
PE: Polyethylene
PFAA: Perfluoroalkyl acid
PFAS: Per- and poly fluoroalkyl substances
PFBA: Perfluorobutanoate
PFBS: Perfluorobutane sulfonate
PFCA: Perfluoroalkyl carboxylates
PFHxA: Perfluorohexanoate
PFHxS: Perfluorohexane sulfonate
PFHxSAm: Perfluorohexane sulfonamido propyl amine
PFHxSAmS: Perfluorohexane sulfonamido propyl quaternary amine
PFOA: Perfluorooctanoate
PFOS: Perfluorooctane sulfonate
PFPeA: Perfluoropentanoate
PFPeS: Perfluoropentane sulfonate
PFSA: Perfluoroalkyl sulfonates
POCIS: Polar organic chemical integrative sampler
TOC: Total organic carbon
TOP: Total oxidizable precursor assay
USGS: United States Geological Survey
WWTP: Wastewater treatment plant

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ABSTRACT

Objectives: The main objective of this project was to better understand factors affecting the mobility and accumulation of poly- and perfluoroalkyl substances (PFAS) in groundwater. Specifically, we investigated how perfluoroalkyl acid (PFAA) precursor transport and biotransformation are affected by varying geochemical conditions in groundwater environments. Our work was conducted at the Joint Base Cape Cod (JBCC) site and surrounding areas that historically received PFAS inputs from Aqueous Film Forming Foams (AFFF) used during fire-training exercises (1970-1985) and a fire emergency in 1997. We leveraged from a collaboration with the U.S. Geological Survey (USGS) for this project, using their detailed understanding of subsurface hydrology to inform our work. Specific objectives of the proposed research were to: (A) Characterize the mobility of PFAA precursors in groundwater environments; (B) Measure biotransformation of major PFAA precursors in AFFF; (C) Formalize understanding of PFAA precursor chemistry in groundwater environments within a model to quantify the mobility of these compounds; (D) Develop and validate passive samplers that can measure time-weighted average concentrations of PFAS.

Technical Approach: Measurements of PFAS composition in field samples indicated 3M AFFF was used approximately 80% of the time at the JBCC field site. Column and biotransformation experiments therefore focused on the PFAS most prevalent in 3M AFFF (sulfonamido precursors and perfluoroalkyl sulfonates [PFSA] with four, six and eight perfluorinated carbons [C4, C6, C8]). Column experiments using sediment and groundwater from JBCC were conducted to determine sediment-water partition coefficients (K_d). Biotransformation experiments were conducted using groundwater-sediment slurries from JBCC representative of the groundwater/surface water boundary to measure biotransformation rates of two major precursors in 3M AFFF (C6 perfluoroalkyl sulfonamido compounds) and an intermediate metabolite (C6 perfluoroalkyl sulfonamide). Soil PFAS concentrations and a decadal record of PFAS concentrations in groundwater at the well closest to one of the Fire Training Areas (FTA-1) at JBCC were used to constrain a dynamic 4-box geochemical model of terminal PFAS and PFAA precursors in groundwater and soil. Microporous polyethylene tubes filled with Oasis hydrophilic lipophilic balance (HLB) sorbent were deployed in the lab and field and tested under different flow conditions, environments and in the presence of biofouling.

Results: Site-specific log K_d values for PFAS in JBCC sediment ranged from -1.7 to 0.6. Lab-derived K_d values agreed with field-derived K_d within the field-derived uncertainty bounds previously reported in Weber et al. (2017) (maximum of an order of magnitude). These ranges were used to parameterize site modeling, and further constrained using a statistical optimization to measured groundwater concentrations. Divalent cation bridging was hypothesized to be the main geochemical mechanism controlling sorption in these low organic carbon sediments.

Approximately 1/3 of the PFAS in 3M AFFF consist of precursors with six perfluorinated carbons (C6) and non-fluorinated amine substituents. Nitrification (microbial oxidation) of amine moieties can transform C6 precursors into perfluorohexane sulfonate (PFHxS). Biotransformation of the most abundant C6 sulfonamido precursors in 3M AFFF with available commercial standards (perfluorohexane sulfonamide [FHxSA] and perfluorohexane sulfonamido propyl tertiary [PFHxSAM] and quaternary [PFHxSAMs] amine) in microcosms representative

of the groundwater/surface water showed rapid biologically associated removal (<1 day) of precursors but slow, microbially-limited biotransformation into PFHxS ($1\text{--}100\text{ pM day}^{-1}$). We propose a transformation pathway that includes one or two nitrification steps and is supported by detection of key intermediates using high resolution mass spectrometry. We found plausible evidence of a link to nitrification that could be explored in future work.

Precursors (mainly C4 and C6 sulfonamido compounds) that can degrade into terminal PFAS accounted for $46\pm 8\%$ of total PFAS (verified using extractable organofluorine analysis) in groundwater at the FTA-1 site. Large temporal variability but no clear temporal trends in PFAS concentrations were apparent over the past decade. Temporal fluctuations for terminal PFAS with fewer precursors (perfluorooctanoic acid: PFOA) and the most hydrophilic PFAS (perfluorobutane sulfonate: PFBS) were significantly correlated with soil moisture ($p < 0.05$), indicating saturation effectively flushes these PFAS through the vadose zone into groundwater. No correlation was observed for other PFAS, including all precursor classes, which have greater propensity for sorption and high surface-activity (higher K_d and K_{ai}). Numerical modeling constrained by measurements suggests that soil contains a much larger reservoir of PFAS than groundwater (2 to 22-fold) and biodegradation of precursors (estimated half-life = 40 – 150 yr) sustains 2021 groundwater concentrations of PFBS (expected mean: 98%, interquartile range [IQR]: 92-99%), and perfluorohexane sulfonate (PFHxS expected mean: 56%, IQR: 19-88%) but not PFOS (expected mean: 0.06%, IQR: 0.01-0.2%).

Tube passive samplers developed as part of this study showed good qualitative agreement between laboratory, field, and model derived measurements.

Benefits: This research develop we developed a combined analytical and statistical method using the total oxidizable precursor assay (TOP) followed by Bayesian inference (TOP + BI) to quantify precursor concentrations grouped by manufacturing origin (ECF versus fluorotelomerization or FT) and perfluorinated carbon chain length (C_n) (Ruyle et al., 2021a, b). This method has the advantage of grouping low concentrations of diverse precursors at AFFF contaminated sites that would be otherwise difficult to detect. TOP+BI explicitly quantifies uncertainty in results introduced by sample extractions, recoveries and oxidation yields following the TOP assay. Using this method, we were able to account for all the PFAS in 3M AFFF (Ruyle 2021a). This was verified by comparing the sum of targeted terminal PFAS and oxidizable precursors from TOP + BI to measured extractable organofluorine (EOF) concentrations, a good proxy for total PFAS in AFFF and predominantly AFFF impacted aqueous samples (Ruyle et al., 2021a, b). This method can also be used to identify the contributions of AFFF to aqueous samples with diverse sources and does not require the use of high-resolution mass spectrometry tools (Ruyle et al., 2021b).

Results of this study suggest treatment of the vadose zone at AFFF contaminated sites will most effectively prevent long-term groundwater contamination. Biotransformation experiments for C6 sulfonamido precursors in 3M AFFF showed a link between transformation and nitrification that could be explored in future site-remediation efforts. A novel tube passive sampler design was developed as part of this study that can provide good time-integrated measurements of nine PFAA. This important because large temporal variability in concentrations at the same groundwater wells were measured over time at JBCC.

EXECUTIVE SUMMARY

Objectives. The main objective of this project was to better understand factors affecting the mobility and accumulation of per- and polyfluoroalkyl substances (PFAS) in groundwater. Specifically, we investigated how perfluoroalkyl acid (PFAA) precursor transport and biotransformation are affected by varying geochemical conditions in groundwater environments. Our work was conducted at the Joint Base Cape Cod (JBCC) site and surrounding areas that historically received PFAS inputs from aqueous film-forming foams (AFFF) used during fire training exercises. The JBCC fire training area sits atop an unconfined aquifer with soils that consist of medium to coarse grain sand and gravel, with extremely low organic carbon (<0.1%), anion exchange capacity, and silt/clay content. We leveraged from decades of U.S. Geological Survey (USGS) research for this project and their detailed understanding of subsurface hydrology of this area. Our work included four main objectives:

- (1) Characterize the mobility of perfluoroalkyl acid (PFAA) precursors in groundwater environments using column experiments;
- (2) Assess factors affecting biotransformation of PFAA precursors and quantify the potential rates of these reactions in natural environments using biotransformation experiments;
- (3) Formalize understanding of PFAA precursor chemistry in groundwater environments using a model to quantify the mobility of these compounds;
- (4) Develop and validate a new tool (passive sampler) for measuring time-weighted average concentrations of PFAS.

Technical Approach. We focused our research on the PFAS most frequently detected at the JBCC field site. The relative abundance of electrochemical fluorination (ECF)-derived precursors in groundwater from JBCC suggested that the use of 3M products constituted approximately 80% of total AFFF use at the site. This result is based on results of the total oxidizable precursor assay interpreted using Bayesian Inference (TOP+BI) in groundwater from JBCC immediately below the former fire-training area. 3M AFFF contains a propriety mixture (Confidential Business Information under the Toxic Substances Control Act) of 850 – 900 mM fluorine as PFAS that is approximately 50% precursors, mainly sulfonamido compounds with 4 and 6 perfluorinated carbons (Barzen-Hansen et al., 2017; Ruyle et al., 2021a).

Since a main driver of PFAS retardation in groundwater is partitioning and sorption to solids, we used column experiments to determine site-specific sediment-water (K_d) partition coefficients. Sediment on Cape Cod, which is primarily quartz and feldspar, has a net negative charge in the range of reported groundwater pH. Partitioning to organic carbon is less important at JBCC compared to other AFFF contaminated sites and ionic interactions with charged sites on sediment surfaces (electrostatic attraction /repulsion) and other ionic co-solvents (ion exchange, divalent cation bridging) likely play a larger role in sorption. A total of 17 PFAS, which include the perfluoroalkyl carboxylates (PFCA) with three through seven perfluorinated carbons (C3 – C7), the C4 –C8 perfluoroalkyl sulfonates (PFSA), the 6:2 and 8:2 fluorotelomer sulfonates (FTSA), and the C4, C6, and C8 perfluoroalkyl sulfonamides (FASA), were chosen for analysis in column experiments because they were the only compounds detected above method detection

limit (MDL = 2.5-40 ng/L) in the groundwater from the fire training area. Sediment and groundwater were collected from the site to perform the column experiments. The hydrodynamics of the columns were tested using bromide as a conservative tracer.

We conducted biotransformation experiments on the most abundant precursors in 3M AFFF with available commercial standards. The most common precursor structures in 3M AFFF are C6 perfluoroalkyl sulfonamido compounds that are expected to biodegrade into perfluorohexane sulfonate (PFHxS). Two commercially available analytical standards exist for precursors in this class (perfluorohexane sulfonamido propyl amine [PFHxSA_m] and perfluorohexane sulfonamido propyl quaternary amine [PFHxSA_mS]) as well as one additional standard for a suspected intermediate metabolite (perfluorohexane sulfonamide [FHxSA]). We incubated water and sediment slurries representative of the groundwater/surface water boundary from the JBCC field site fortified with diluted concentrations of individual precursor spikes or 3M AFFF and subsampled at defined intervals. Experiments were performed in triplicate and abiotic controls were included in each experiment.

We analyzed archived groundwater samples ($n=191$ samples) from the plume originating from the JBCC fire training area (FTA-1) collected between 2002 and 2021. PFAS concentrations showed substantial variability in the downstream plume, and we determined that additional sampling would be needed to fully define the plume position and extent of the contamination. We therefore refined the spatial domain of our modeling to focus on PFAS transport and transformation in the immediate vicinity of the fire training area, where abundant data for the well immediately below the fire-training area were available from archival USGS samples and data reported by the Air Force Civil Engineering Center (AFCEC) and the National Institute of Standards and Technology (NIST).

PFAS precursors released to the environment may undergo abiotic or biotic transformation and eventually form perfluoroalkyl acids (PFAA) as terminal products. Precursors that originate from the electrochemical fluorination (ECF) process have a fully fluorinated backbone in their chemical structure, while those manufactured by the fluorotelomerization (FT) process are not fully fluorinated (Buck et al., 2011). Targeted mass spectrometry methods (LC-MS/MS) only capture a small fraction of the PFAS used in commerce and released to the environment (Yeung and Mabury, 2016; Koch et al., 2019). It is challenging to detect most precursors using targeted methods because many analytical standards are not commercially available. High-resolution mass spectrometry (HRMS) can be used to confirm the presence of specific precursors and assign probable structures to unknown PFAS. However, these results are not quantitative and are difficult to interpret when diverse precursors are present at low concentrations, which is often the case with AFFF contaminated systems. Semi-quantification of PFAS from HRMS measurements has been used to estimate the concentrations of analytes that lack matched analytical standards, but uncertainties are not quantifiable and could span an order of magnitude or more (Nickerson et al., 2021; Charbonnet et al., 2021).

To address some of the challenges associated with PFAS precursor detection, we developed a statistical method for interpreting results from the total oxidizable precursor (TOP) assay that groups precursors by their perfluorinated carbon chain length and manufacturing origin (ECF or FT) using Bayesian inference (TOP+BI)

(<https://github.com/SunderlandLab/oxidizable-pfas-precursor-inference>; Ruyle et al., 2021a).

The TOP assay transforms oxidizable precursors to perfluoroalkyl carboxylates (PFCA) with known perfluorinated carbon chain lengths that are detectable at trace levels using targeted LC-MS/MS analysis. The TOP+BI method is preferred over analytically detected changes in PFCA concentrations (only TOP) because it explicitly accounts for analytical uncertainties, incomplete recoveries, and variability in product yields following precursor degradation. This toolbox can quantify all PFAS originating from legacy ECF AFFF produced by 3M (validated using EOF measurements) and was applied in this study to samples collected for more than a decade from immediately below the fire training area.

We developed a 4-box geochemical model to represent the temporal evolution of precursors and changes in reservoirs of the terminal PFAS in soils and groundwater at the former fire-training area over time. The model includes individual compartments for precursors and terminal compounds in soil and groundwater. It is forced by inputs from AFFF-use, constrained by known dates of use and measurements of PFAS in soil cores at the fire-training area, and in groundwater over time. We used uniform prior probability distributions to characterize plausible uncertainty of AFFF use (PFAS loading), precursor biodegradation rates, and retardation due to partitioning to solids and retention at the air-water interface using data obtained from the literature (Weber et al., 2017; Guo et al., 2020) and column experiments as part of this study. Advection rates through soil and groundwater were based on hydrological information from USGS. We then optimized the simulation (PFAS loading, biodegradation rate, and retardation) to reproduce measured concentrations by selecting parameter values within their uncertainty ranges that result in the smallest differences between modeled and observed soil and groundwater concentrations using Bayesian inference. Specifically, we sample from the data-optimized distribution of parameters using Markov Chain Monte Carlo (MCMC) simulations with the same sampler and hyperparameters that were used to infer oxidizable precursor concentrations from the TOP assay (see Ruyle et al., 2020a). We calculated site specific sediment-water partition coefficients (K_d) and air-water interface partition coefficients (K_{ai}) from inferred retardation factors using the equations derived in Brusseau et al. (2018).

A novel tube passive sampler was developed using microporous polyethylene tubes filled with Oasis hydrophilic lipophilic balance (HLB) sorbent. Samplers were deployed in the lab and field and tested under different flow conditions, environments and in the presence of biofouling. In the lab, triplicate tube passive samplers were deployed at four different flow rates (0, 10, 20, and 60 cm s⁻¹) and two different temperatures (15°C and 25°C). Passive samplers were deployed in the field for 14-28 days at three locations, including AFFF-impacted groundwater (JBCC) and river (QR9) downstream of a former fire training area on Joint Base Cape Cod, MA and a river downstream of a historical textile mill in Westerly, RI (Pawcatuck). Uptake of nine PFAA was compared under experimental tank deployments, field deployments on Cape Cod, MA, and to a numerical model for integrative passive sampling from the literature (Schaefer et al, 2019; Vrana et al., 2005) that was customized to predict sampling rates (mL day⁻¹) from the physicochemical properties of PFAS and the passive sampler design.

Results. Results of column experiments showed site-specific log K_d values for PFAS in Cape Cod sediment ranged from -1.7 to 0.6. Each additional perfluorinated carbon was associated with a 0.72 ± 0.05 increase in log K_d averaged across all PFAS classes. For the same number of

perfluorinated carbons, sorption also depended on the functional group and followed the order PFCA < PFSA < FTSA < FASA. Lab-derived K_d values agreed with field-derived K_d reported by Weber et al. (2017) within an order of magnitude. A spike in aqueous-phase concentration due to desorption was observed for the anionic PFAS with $\log K_d$ values > -1 during elution with PFAS-free groundwater with much lower Ca^{2+} concentrations. This likely reflects an important role for divalent cation bridging on sorption in these low organic carbon sediments. Carboxylates exhibit greater complexation than sulfonates to divalent cations such as Ca^{2+} and Fe^{2+} due to their lesser degree of hydration (Groenedijk et al., 2021). We hypothesize that cation bridging could explain field data from a nearby kettle lake that showed substantially greater sorption of PFCA compared to PFSA in infiltrating groundwater under reducing conditions, which produce divalent Fe^{2+} and Mn^{2+} (Tokranov et al., 2021).

For the three C6 sulfonamido precursors included in this study (PFHxSAm, PFHxSAmS, FHxSA), the expected means of rate constants for microbial association exceeded sorption by at least a factor of 2x (Table 25) and ranged between -0.2 to -5.0 days⁻¹. At 29°C, the first order half-life of PFHxSAmS and PFHxSAm was less than half a day and approximately 5x faster than the bioassociation of FHxSA (Table 25). Biotransformation of all three precursors was indicated by formation of PFHxS, the terminal product of microbial biotransformation of C6 sulfonamido precursors (**Figure 18**). Formation of PFHxS appeared to proceed at a constant rate throughout the experiment regardless of any changes in the concentrations of reactants (precursors). These results imply that the formation of PFHxS was not limited by the supply of precursors for biotransformation but rather by the microbial reactions catalyzing this transformation. Despite faster microbial association of the zwitterions compared to FHxSA, the rate limiting step for PFHxS production is the oxidation of the zwitterions to FHxSA rather than oxidation of FHxSA to PFHxS. This could explain why minimal accumulation of FHxSA was observed in the bioactive experiments. The relative stability of precursors against oxidation into PFAA in soils allows for their transport through the vadose zone into groundwater and downstream watersheds (Weber et al., 2017). Past work near the field sampling location for the groundwater/sediment slurries used in this study showed a distinct seasonality in precursor transport across the groundwater/surface water boundary immediately downgradient from the legacy AFFF plume (Ashumet Pond, MA) (Tokranov et al., 2021). Rapid removal (<36 hours) of up to 85% of precursors was observed in field data during the summer months but not in the winter months. These field-observations are consistent with the large and rapid (<1 day) bioassociation of precursors observed experimentally in this study (Figure 16). We estimated based on modeling that biotransformation of all precursors into PFHxS would require more than a year (Figure 20). These results suggest that some of the precursors and/or intermediate metabolites associated with the immobile microbiome in the field will be released back into the aqueous phase during winter months when turnover of microbial biomass occurs, which is consistent with prior field observations (Tokranov et al., 2021).

In groundwater samples from below the JBCC fire training area, all EOF (101±7%) was accounted for by combining targeted PFAS measurements with the TOP assay interpreted using Bayesian inference (Figure 22). The C6 and C8 PFSA and the C7 PFCA were the predominant terminal PFAS, consistent with their composition in ECF AFFF (Ruyle et al. 2021a; Houtz et al. 2013). High resolution mass spectrometry yielded unambiguous spectrum-structure matches for 15 precursors, 9/15 of which were C4 and C6 perfluoroalkyl sulfonamido compounds that have

either been previously identified in 3M AFFF or are expected biodegradation intermediates. Large temporal variability but no clear temporal trends in PFAS concentrations was observed between 2007 and 2021. Temporal fluctuations for terminal PFAS with fewer precursors (perfluorooctanoic acid: PFOA) and the most hydrophilic PFAS (perfluorobutane sulfonate: PFBS) were significantly correlated with soil moisture ($p < 0.05$), indicating saturation effectively flushes these PFAS through the vadose zone into groundwater. No correlation was observed for other PFAS, including all precursor classes, that have greater propensity for sorption and high surface-activity (high K_d and K_{ai}). Numerical modeling constrained by measurements suggested that soil contains a much larger reservoir of PFAS than groundwater (2–22-fold), and biodegradation of precursors (estimated half-life = 40 – 150 yr) is the greatest contributor (expected mean 56–98%) to 2021 concentrations of PFBS and PFHxS in groundwater below the former fire-training area (Figure 27).

Results of passive sampler deployments showed that at 15 °C, the measured sampling rates for perfluorohexanoic acid (PFHxA) of 48–76 mL day⁻¹ were in good agreement with the model predictions (51 mL day⁻¹) across 10–60 cm s⁻¹ water flow speeds. PFHxS sampling rates at 15 °C were in similar agreement (11 +/- 6 mL day⁻¹ measured, 14 +/- 4 mL day⁻¹ modeled) and displayed no discernible increase across variable water flows. Similar sampling rates were observed during field deployments, highlighting the general applicability of the sampler in environmental conditions. This research demonstrates that the polyethylene tube's sampling rates are predictable given a known temperature across a range of flow speeds, with no discernable impacts of biofouling.

Benefits. This research highlights the potential role of divalent cation bridging at field-sites with low organic carbon, anion exchange, and silt/clay content for retarding PFAS transport. Biotransformation experiments show precursors are strongly bio-associated with the microbiome but slowly transformed. Seasonal fluctuations in the microbiome may be an important factor dictating precursor transport in groundwater and at the groundwater/surface water boundary. The statistical inference procedure developed as part of this work differentiates the relative use of ECF versus fluorotelomer (FT) AFFF in the environment (Ruyle et al. 2021b), which is particularly useful at military fire training areas where both types of products were often used but poor record keeping prevents a priori knowledge of how much of each type of AFFF was used (Place and Field, 2012). Field data and modeling results suggest treatment of soil at AFFF contaminated sites will most effectively prevent long-term groundwater contamination. A novel tube passive sampler design was developed as part of this study that can provide good time-integrated measurements of nine PFAA. This is important because large temporal variability in concentrations at the same groundwater wells were measured over time at JBCC.

PROJECT OBJECTIVES AND TASKS

The overarching objective of this proposal was to better understand factors affecting the mobility and accumulation of per- and polyfluoroalkyl substances (PFAS) in groundwater. Specifically, we characterized how perfluoroalkyl acid (PFAA) precursor transport and biotransformation are affected by varying environmental conditions. Our work was conducted at the Joint Base Cape Cod (JBCC) site and surrounding areas that historically received PFAS inputs from Aqueous Film Forming Foams (AFFF) used for fire-training and fire-fighting activities. We leveraged from a collaboration with the U.S. Geological Survey (USGS) for this project, using their detailed understanding of subsurface hydrology of this area and geochemical expertise to inform our work.

To achieve these goals, this project focused on four specific tasks within the context of the JBCC field-site:

- 1. Characterize the mobility of perfluoroalkyl acid (PFAA) and precursors in groundwater environments and interactions with geochemical conditions.**
- 2. Assess factors affecting biotransformation of PFAA precursors and quantify the potential rates of these reactions in natural environments.**
- 3. Develop a model for formalizing understanding of PFAA and precursor mobility in groundwater environments.**
- 4. Develop and field test passive samplers that measure time-weighted average concentrations of PFAS.**

This final report for SERDP ER18-1280 describes the activities and results associated with:

Task 1: PFAA and precursor mobility in groundwater

Task 2: Precursor biotransformation

Task 3: JBCC fire-training area model, and

Task 4: Development and testing of passive samplers.

Completion of these tasks addresses the Statement of Need (ERSON-18-C1: Improved Understanding of Per- and Polyfluoroalkyl Substance Source Zones) because our work adds to knowledge on the behavior of AFFF source zones and PFAS retardation mechanisms, fills gaps in understanding of biotransformation of AFFF in source zones, develops a model for PFAS fate and impacts, and new tools for characterizing source zone properties.

BACKGROUND

Field site: As described in Weber et al. (2017), the field site and focus of this research (FTA-1) is located on western Cape Cod, Massachusetts (**Figure 1**), at the USGS Cape Cod Toxic Substances Hydrology Research Site located on the Joint Base Cape Cod (JBCC).

The site has been the subject of long-term research on the fate and transport of contaminants in groundwater, and the hydrogeology and biogeochemistry of the aquifer have been extensively characterized. The fire training area at JBCC (FTA-1) was used from 1958 to 1985, and records indicate that jet fuel, chlorinated hydrocarbons, transformer oils, paint thinners, and gasoline were utilized (AFCEE/MMR 1999). Use of AFFF at FTA-1 likely started in 1970 and continued until 1985, with a singular additional application in 1997. Thermal soil remediation targeting fuel constituents and chlorinated solvents was undertaken at FTA-1 in 1997 when soil was excavated to a maximum depth of 11 m below ground surface and heated to between 157 °C and 204 °C before being backfilled. Because PFOA is thermally stable up to 300 °C, and perfluorinated sulfonates require even higher temperatures for thermolysis (Glöckner et al., 1989; Wang et al., 2011), the soil treatment was unlikely to have reduced concentrations of PFAA. However, some PFAA precursors may have been thermolyzed to terminal PFAA.

FTA-1 is located 580 m upgradient of a wastewater treatment plant (WWTP) where secondary treated effluent was disposed to groundwater through rapid-infiltration beds (referred to herein as infiltration beds) from 1936 to 1995 (**Figure 1**). Boron is used as an indicator for the wastewater plume location (**Figure 1**). Wastewater effluent disposal to the aquifer resulted in a large and chemically complex contaminant plume characterized by low dissolved oxygen (DO) concentrations and elevated concentrations of dissolved organic carbon (DOC), nitrate, phosphate, ammonium, boron, and organic micropollutants (Repert et al., 2006; Parkhurst et al., 2003; Barber et al., 1988).

The glacial outwash sediments that comprise the unconfined aquifer at the study site consist of medium to coarse sand and gravel. Hydraulic properties measured during a tracer experiment at the site include a hydraulic conductivity of 110 m d⁻¹, porosity of 0.39, and average flow velocity of 0.42 m d⁻¹ (Garabedian et al., 1991; LeBlanc et al., 1991). Recharge to the aquifer is 73 cm yr⁻¹ (Walter et al., 2016). The water table near FTA-1 generally slopes (hydraulic gradient of 0.0015) southeastward toward Ashumet Pond, a glacial kettle pond, and southward toward Nantucket Sound (LeBlanc et al., 1984). The water table is about 17 m and 6 m below land surface at FTA-1 and the infiltration beds, respectively.”

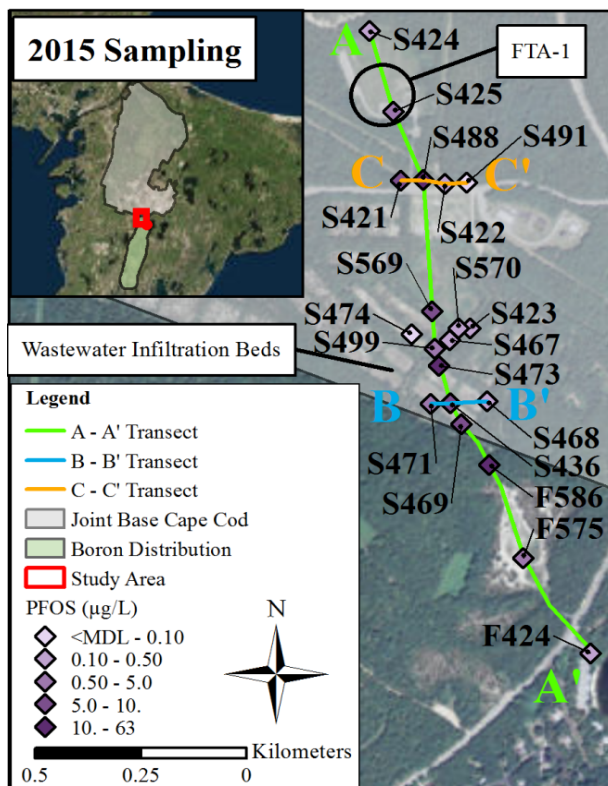


Figure 1. Locations of groundwater sampling monitoring for PFAS.

Map shows sampling locations prior to start of this study (diamond symbols) at the former fire training area (FTA-1) FTA-1 on the Joint Base Cape, Cod (JBCC), MA USA Symbol shading corresponds to the maximum concentration of perfluorooctane sulfonate (PFOS). Figure from Weber et al. (2017).

Task 1 Background: PFAS and precursor mobility in groundwater

The main driver of PFAS retardation in groundwater is partitioning and sorption to solids. Sediment-water partitioning of PFAS is controlled by their ionic and hydrophobic chemistries (Higgins and Luthy, 2006; Nguyen et al., 2020). Previous work has demonstrated that hydrophobic partitioning into organic carbon on solid surfaces is the dominant sorption mechanism in sediment with high fractions of organic carbon ($> 0.1\%$) (Higgins and Luthy, 2006; Umeh et al., 2021). Partitioning to organic carbon leads to distinct headgroup and chain length patterns. Sorption is greatest for perfluoroalkane sulfonamides (FASAs), intermediate for perfluoroalkyl sulfonates (PFSAs), and lowest for perfluoroalkyl carboxylates (PFCAs) and increases with the number of perfluorinated carbons (Guelfo et al., 2013; Higgins and Luthy, 2006; Nguyen et al., 2020). However, partitioning to organic carbon is less important at our field site on Cape Cod due to low sediment organic carbon ($< 0.01\%$) (Barber et al., 1992). The lack of organic carbon sites results in lower sediment-water partition coefficients (K_d) at the field site (Weber et al., 2017) compared to those reported in the literature (Guelfo et al., 2013; Higgins and Luthy, 2006; Nguyen et al., 2020; Umeh et al., 2021).

Ionic interactions with charged sites on sediment surfaces (electrostatic attraction/repulsion) and other ionic co-solvents (ion exchange, divalent cation bridging) likely to play a larger role in sorption at the field site (Du et al., 2014; Li et al., 2018). Sediment on Cape Cod, which is primarily quartz and feldspar, has a net negative charge in the range of reported groundwater pH (Barber et al., 1992). Positively charged surface sites include iron and aluminum oxyhydroxides and make up $< 25\%$ of the sediment surface (Barber et al., 1992), but control the mobility of other contaminants at the site (Kent et al., 2000; 2007). Previous studies have shown that pH, ionic strength, anion exchange capacity, and divalent cations such as Ca^{2+} influence ionic PFAS sorption (Campos-Pereira et al., 2020; Du et al., 2015; Higgins and Luthy, 2006; Johnson et al., 2007; Li et al., 2018; Nguyen et al., 2020; Umeh et al., 2021). Sorption of PFAS typically increases with acidity as more sites on sediment surfaces become positively charged (Campos-Pereira et al., 2020; Du et al., 2015; Higgins and Luthy, 2006; Li et al., 2018). Sorption also increases with ionic strength due to salting out and anion exchange capacity (Nguyen et al., 2020; Munoz et al., 2017). However, these processes are not likely important at the field site due to low overall ionic strengths (specific conductivity $< 400 \mu\text{S cm}^{-1}$) (Weber et al., 2017) and negligible anion exchange capacity across a broad range of environmentally relevant pH (

Table 1). Complexation of PFAS with divalent cations such as Ca^{2+} and a negatively charged sediment surface site enhances sorption (Higgins et al., 2006; Du et al., 2014; Li et al., 2018). Cation concentrations vary over orders of magnitude at the field site (

Table 1) and play an important role in PFAS sorption.

Table 1. Water quality parameters for groundwater used in column experiments.

	FTA Groundwater	Reference groundwater
<i>PFAS</i>		
Perfluorobutanoate: PFBA [ng L ⁻¹]	140	0
Perfluoropentanoate: PFPeA [ng L ⁻¹]	350	0
Perfluorohexanoate: PFHxA [ng L ⁻¹]	500	0
Perfluoroheptanoate: PFHpA [ng L ⁻¹]	155	0
Perfluorooctanoate: PFOA [ng L ⁻¹]	760	0
Perfluorobutane sulfonate: PFBS [ng L ⁻¹]	90	0
Perfluoropentane sulfonate: PFPeS [ng L ⁻¹]	115	0
Linear perfluorohexane sulfonate: PFHxS [ng L ⁻¹]	1750	0
Branched PFHxS [ng L ⁻¹]	275	0
Perfluoroheptane sulfonate: PFHpS [ng L ⁻¹]	65	0
Linear PFOS [ng L ⁻¹]	1200	0
Branched PFOS [ng L ⁻¹]	580	0
6:2 fluorotelomer sulfonate: FTSA [ng L ⁻¹]	250	0
8:2 FTSA [ng L ⁻¹]	175	0
Perfluorobutane sulfonamide: FBSA [ng L ⁻¹]	70	0
Perfluorohexane sulfonate: FHxSA [ng L ⁻¹]	200	0
Perfluorooctane sulfonamide: FOSA [ng L ⁻¹]	35	0
<i>Water quality parameters</i>		
pH	6.15	5.04
Specific conductivity [μ S cm ⁻¹]	123	38
Salinity [ppt]	0.1	0.02
Dissolved organic carbon: DOC [mg L ⁻¹]	1.8	1.3
Chloride [mg L ⁻¹]	15.4	7.7
Nitrate [mg L ⁻¹]	9.9	0.2
Sulfate [mg L ⁻¹]	14.4	2.7
Bromide [mg L ⁻¹]	0.1	0.0
Lithium [mg L ⁻¹]	0.0	0.0
Sodium [mg L ⁻¹]	10.0	4.1
Ammonium [mg L ⁻¹]	0.0	0.0
Potassium [mg L ⁻¹]	1.2	0.3
Calcium [mg L ⁻¹]	7.9	0.8
Magnesium [mg L ⁻¹]	2.6	1.1
Sum of divalent cations [mg L ⁻¹]	10.5	1.9

Task 2 Background: Precursor biotransformation

The potential production of PFAA after AFFF use through biodegradation of precursors is substantial because both legacy and contemporary AFFF contain large amounts of precursors ($\geq 50\%$ of total PFAS) (Houtz et al., 2013; Ruyle et al., 2021a). Short chain precursors with four to six (C4-C6) perfluorinated carbons are much more abundant than their terminal PFAA in these products ($> 7x$) (Ruyle et al., 2021a). Our work has estimated that most of the AFFF released at the JBCC field site was legacy products produced by 3M through the electrochemical fluorination (ECF) process (Ruyle et al., 2021b). The most common precursor structures in 3M AFFF are C6 perfluoroalkyl sulfonamido compounds that are expected to biodegrade into perfluorohexane sulfonate (PFHxS) (Houtz et al., 2013; Barzen-Hanson et al., 2017). Two commercially available analytical standards exist for precursors in this class (perfluorohexane sulfonamido propyl amine [PFHxSAm] and perfluorohexane sulfonamido propyl quaternary amine [PFHxSAmS]) as well as one additional standard for a suspected intermediate metabolite (perfluorohexane sulfonamide [FHxSA]).

To date, no studies have explored the biodegradation of the C6 perfluoroalkyl sulfonamido compounds found in AFFF (Choi et al., 2022). Prior work has examined the biodegradation of perfluorooctanoate (C7: PFOA) and perfluoroalkyl sulfonate (C8: PFOS) precursors that might provide insight into analogous mechanisms for C6 compounds (Mejia-Avendaño et al., 2016; Chen et al., 2020; Liu et al., 2021). Parent precursors can be quickly degraded into intermediate metabolites in aerobic soil microcosms in multistep reactions that involve deacetylation, decarboxylation, N-demethylation, and oxidation (Chen et al., 2020; Liu et al., 2020). However, the yields to their terminal compound (PFOA and PFOS) are typically low ($<3\%$) in the months that these experiments are typically conducted (Mejia-Avendaño et al., 2016; Chen et al., 2020; Liu et al., 2021). Outside of AFFF soil microcosms, biodegradation of perfluoroalkyl sulfonamido precursors has been observed in wastewater sludge and wastewater treatment plants (Rhoads et al., 2008; Lange 2018). These studies show that the production of terminal PFAA is rate limited by the biodegradation of the perfluoroalkyl sulfonamide (FASA). Field studies have also shown high concentrations of FASA, particularly FHxSA, in AFFF-impacted soil and groundwater, suggesting this class semi-persistent in the environment (Houtz et al., 2013; Mejia-Avendaño et al., 2016; Nickerson et al., 2020).

Task 3 Background: JBCC fire-training area model

PFAS remain in soil and groundwater decades after AFFF use (Houtz et al., 2013; Weber et al., 2017; Nickerson et al., 2020). Their long-term persistence is controlled by the biodegradation of precursors and sequestration in immobile phases (sediment, non-aqueous phase liquids) and boundaries (soil air-water interface) (Brusseau et al., 2018). Theory and controlled laboratory studies have revealed that long chain PFAA such as PFOS can be retained in unsaturated soil for decades due to their large surface activity and propensity to sorb onto soil/sediment surfaces (Guo et al., 2020; Zeng et al., 2021). However, there are discrepancies in the fate of short chain PFAA between lab studies and field studies. Lab studies show minimal surface activity and affinity to sorb onto solids (Vecitis et al., 2008; Campbell et al., 2009; Guelfo and Higgins, 2013). By contrast, field campaigns show persistent and elevated concentrations of compounds such as PFBS and PFHxS (Houtz et al., 2013; Weber et al., 2013; Nickerson et al., 2020), suggesting the presence of unresolved mechanisms for these PFAS. We hypothesized that biodegradation of abundant (Houtz et al., 2013; Ruyle et al., 2021a) and more sorptive (Nguyen et al., 2020; Schaefer et al., 2021) PFBS and PFHxS precursors in AFFF sustain their elevated measured concentrations at the JBCC field site.

Modeling is a useful approach for evaluating competing biogeochemical mechanisms such as precursor biodegradation, air-water interfacial partitioning, and soil/sediment sorption. Modeling informed by data from a field site over time can integrate real-world dynamic variability in these mechanisms due to changes in time variable hydrology (such as soil saturation) and aqueous chemistry. Modeling provides complementary insights to intensive field campaigns that directly measure sorption/desorption constants (Schaefer et al., 2021) and soil-to-groundwater fluxes using lysimeters (Anderson et al., 2021), but are temporally limited due to the logistics and costs associated with subsurface sampling.

Here, we leverage long-term archival samples from the JBCC field site (between 2002 and 2021) collected by the USGS to establish the first multi-decade record of PFAS contamination at an AFFF-contaminated site. We use these data, along with the site's well characterized hydrogeology, to develop a box model for PFAS fate in soil and groundwater at the fire training area. We use a box modeling approach to evaluate the role of precursor biodegradation, air-water interfacial partitioning, and soil/sediment sorption for four representative PFAA (PFBS, PFHxS, PFOS, and PFOA) and their precursors after AFFF use.

Task 4 Background: Passive samplers

Passive sampling is a low cost, low maintenance alternative to traditional water grab sampling. Integrative passive samplers report time weighted averages of water concentrations that can provide insight into surface water contamination across diurnal, tidal, and seasonal trends than discrete grab sampling. The unique chemistry of PFAS makes most traditional non-polar passive sampling technology not applicable for monitoring ionic PFAS. This creates the distinct need for affordable, reliable, and versatile detection tools. This task was performed to calibrate and validate a passive sampler based on a microporous polyethylene tube sampler filled with sorbent for PFAS in surface water.

Existing passive samplers for PFAS are typically operating in the linear uptake mode as integrative samplers, such as the polar organic chemical integrative sampler (POCIS) design (Harman et al., 2011; Fauvelle et al., 2017; Kaserzon et al. 2012; 2013; Lai et al., 2019). Other diffusion-based sampler designs such as the diffusive gradient in thin film (DGT) samplers have been investigated for PFAS in surface waters, with initial research done on diffusion of PFAS through the DGT's membrane (Fang et al., 2021; Gobelius et al., 2019; Urik and Vrana, 2019; Wang et al., 2021). Recently, an equilibrium-based sampler for PFAS was investigated, using modified nanographene hydrogel to create a water-based sampler as opposed to sorbent-based sampler (Becanova et al., 2021).

This study was focused on the validation of a tube passive sampler that aims to minimize the impact of flow on integrative passive sampler uptake by using a much thicker membrane than previously designed samplers for PFAS. Using a design first introduced for passive sampling of glyphosate, aminomethyl phosphonic acid (AMPA), and PFAS in groundwater, this tube passive sampler possesses a 0.2 cm thick microporous polyethylene membrane in contrast to the 0.014 cm thick membranes reported for POCIS deployments (Fauvelle et al., 2017; Kaserzon et al., 2019; Booij et al., 2020).

Equation 1 ascribes the overall mass transfer estimation (k_0) of a polar organic compound into a sorbent filled membrane through the summation of three main phases: mass transfer through the water boundary layer (k_w), transfer through the membrane (k_m), and finally through the sorbent (k_s) with units of velocity for all three transfers:

$$\frac{1}{k_0} = \frac{1}{k_w} + \frac{1}{k_m K_{mw}} + \frac{1}{k_s K_{sw}} \quad (\text{Equation 1})$$

Where K_{mw} is the dimensionless partitioning constant for a given compound between the membrane and water, and K_{sw} is the dimensionless partitioning between the sorbent and water for a given compound (Booij et al., 2007; Kaserzon et al., 2012; Vrana et al., 2007; Stephens et al., 2005).

This uptake rate model can be adjusted to reflect the unique chemistry of each PFAS and the properties of the microporous membrane and sorbent used in the novel tube passive sampler for PFAS. Using an estimation for the k terms from literature the equation can be modified to

calculate mass transfer in terms of aqueous diffusion through the water boundary layer, the pore space, and the sorbent pack as water passes through the sampler (Equation 2)

$$\frac{1}{k_0} = \frac{1}{k_w} + \frac{1}{K_{mw}*(D_w*p_m/d_m)} + \frac{1}{K_{sw}*(D_w*p_s/d_s)} \quad (\text{Equation 2})$$

Where: D_w is the aqueous diffusion of a PFAS compound ($\text{cm}^2 \text{s}^{-1}$),
 p is the porosity of the membrane and sorbent (%), and
 d (cm) is the thickness of the membrane (d_m) and sorbent layer (d_s) (Vrana et al., 2005).

With this model, the sampling rate, a vital parameter for integrative passive sampling, can be predicted, and investigated across many environmental parameters including water flow speed, water temperature, water viscosity, as well as physical parameters associated with sampler designs including membrane types and different sorbent choices using Equation 3 where A (cm^2) is the total surface area of the novel tube passive sampler:

$$\frac{1}{R_s} = \frac{1}{k_0*A} \quad (\text{Equation 3})$$

Sampling rate (R_s), with units of milliliters per day, can then be used as the conversion between mass of a given PFAS compound accumulated within the sampler to the time weighted average concentration in the water during the deployment length (Equation 4):

$$C_w = \frac{m_s}{R_s*t} \quad (\text{Equation 4})$$

Where C_w is the time weighted average water concentration (ng L^{-1}),
 m_s is the mass of a PFAS compound measured in the sampler (ng), and
 t is the length of the deployment, in days.

Equation 4 can also be rearranged to calculate a sampling rate for experimentally deployed passive samplers with known water concentrations in a laboratory setting. The use of this 3-layer adjusted model can be compared to a simpler version of the model that has been highlighted for use with POCIS samplers in the literature which de-emphasizes the impact of sorbent on the overall uptake kinetics (Equation 5) (Booij et al., 2020).

$$\frac{1}{R_s} = \frac{1}{k_w*A} + \frac{1}{R_{s,max}} \quad (\text{Equation 5})$$

Where $R_{s,max}$ (mL day^{-1}) is a theoretical value that accounts for the maximum sampling rate that could be achieved by diffusion through the water boundary layer and the membrane of the passive sampler in question.

Results from Equation 5 can be used when transport resistance in the sorbent can be ignored, and transfer is entirely through the pore space (Booij et al., 2020). The maximum sampling rate is derived from the physico-chemical parameters of the individual PFAS compound interacting with the membrane (Equation 6).

$$R_{s,max} = \frac{D_w * \emptyset}{\tau^2 * d} * A \quad (\text{Equation 6})$$

Where: $\emptyset$ is the porosity of the membrane,
 d is the membrane thickness (cm), and
 τ is the tortuosity of the flow path through the pore space, which is always assumed to be 1 (Booij et al., 2020).

Most prior work reported calculated sampling rates for PFAS by POCIS sampler, as opposed to other emerging contaminants (Gobelius et al., 2019; Booij et al., 2020). The use of a thick, polyethylene membrane could then be a favorable alternative to previously tested passive samplers, with a lower partitioning PFAS to the membrane, an increased duration of the linear uptake phase and a reduced flow dependency (Endo et al., 2019).

In summary, the purpose of this task was to (i) validate the uptake of PFAS in the tube passive sampler in a tank under controlled laboratory conditions (ii) investigate the impacts of flow, temperature, and biofouling on the measured and modeled sampling rates of selected PFAS, and (iii) assess the performance of the passive sampler in deriving TWA concentrations of PFAS in field deployments.

MATERIALS AND METHODS

Task 1 Methods: PFAA and precursor mobility in groundwater

Sample collection and characterization. Column experiments were performed to determine site specific sediment-water (K_d) partition coefficients. Sediment was collected on 12 March 2019, at a depth of 1.3 – 1.6 meters below land surface at the USGS Toxic Substance Hydrology Research Program’s field station downgradient of the former fire training area on Cape Cod, Massachusetts (**Figure 2**).

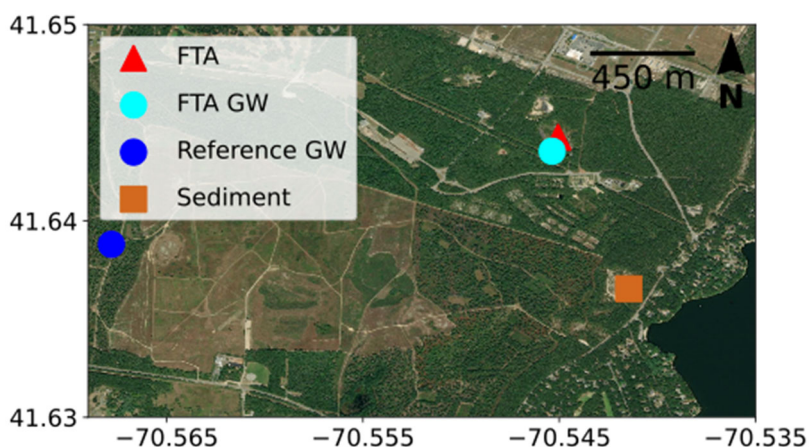


Figure 2. Map of field site on Cape Cod, Massachusetts, United States.

Origins of sediment and groundwater used in the column experiments are shown by symbols

Prior work shows that sediment at this depth has the same composition as deeper saturated-zone sediment (Stollenwerk, 1995). All field equipment and sampling containers were cleaned for trace-metals and PFAS twice with Milli-Q water, once with 10% v:v nitric acid (J.T. Baker, Radnor, Pennsylvania), once with LCMS-grade methanol (J.T. Baker, Radnor, Pennsylvania), twice with Milli-Q water, and air dried. Sediment was collected using a stainless-steel hand trowel in 4 L glass jugs with polyethylene caps (VWR International; Radnor, Pennsylvania), stored on ice in the field, and transferred into a -20°C freezer within four hours of collection. Prior to sieving, the sediment was thawed and air dried in clean polypropylene vats lined with aluminum foil. The sediment was sequentially sieved through a 1400 and 500 μm mesh. Sieved sediment was stored dry at room temperature prior to the experiment.

The sediment passed through both meshes ($< 500 \mu\text{m}$ diameter) represented 25% of the total volume collected in the field. Sediment $< 500 \mu\text{m}$ diameter at this site is primarily comprised (75 – 95%) of quartz (SiO_2 ; $\text{pH}_{\text{PZC}} = 2$) and feldspar ($(\text{Na,Ca,K,Ba})\text{AlSi}_3\text{O}_8$; $\text{pH}_{\text{PZC}} = 1.8$) (Barber et al., 1992). Metallic mineral species, which comprise $< 25\%$ of the sediment, include glauconite ($(\text{K,Na})(\text{Fe}^{3+},\text{Al,Mg})_2(\text{Si,Al})_4\text{O}_{10}(\text{OH})_2$; $\text{pH}_{\text{PZC}} = 6.5$), goethite ($(\text{FeO}(\text{OH}))$; $\text{pH}_{\text{PZC}} = 7.8-8.3$), and biotite ($(\text{K}(\text{Mg,Fe})_3(\text{AlSi}_3\text{O}_{10})(\text{F,OH})_2$; $\text{pH}_{\text{PZC}} = 6-7$) (Barber et al., 1992).

The fraction of organic carbon was less than 0.03% (Weber et al., 2017). Sediment $\text{pH}_{\text{BaCl}_2}$, anion exchange capacity, and cation exchange capacity were measured following the method of Gillman and Sumpter (1986). $\text{pH}_{\text{BaCl}_2}$ was 5.40 ± 0.02 ($n = 6$). Anion exchange capacity [$\text{cmol}(\text{e}^-) \text{kg}^{-1}$] and cation exchange capacity [$\text{cmol}(\text{p}^+) \text{kg}^{-1}$] were measured at pH between 2.35 and 6.5. Cation exchange capacity did not vary with pH ($3.39 \pm 0.01 \text{ cmol}(\text{p}^+) \text{kg}^{-1}$). Anion exchange capacity was less than 0 for $\text{pH} \geq 4.2$ and was $11,300 \text{ cmol}(\text{e}^-) \text{kg}^{-1}$ for $\text{pH} = 2.35$.

Groundwater was collected from the fire training area [USGS well MA-SDW 425-0063 (30MW0417C)] and a PFAS-free reference site (USGS well MA-FSW 605-M01-04BUT) (Figure 1) on July 20, 2021. Samples were collected using a peristaltic pump, stored on ice in the field, and transferred into a 4°C refrigerator prior to the experiment. The FTA groundwater was sampled from the uppermost 2.2 m of the water table (17.6 - 19.9 m below ground surface). The PFAS-free reference site was sampled from 0.08 m below the water table (13.9 m below ground surface). Water quality parameters of the two groundwater sampling locations (Table 1) were measured in the lab immediately prior to the experiment. Data for pH, specific conductivity, and salinity were measured using a YSI Professional Plus Multiparameter probe (YSI Inc., Yellow Springs, Ohio). DOC was measured using a total organic carbon analyzer (Shimadzu, Kyoto, Japan). Anions and cations were measured on a Metrohm 920 Compact IC Flex ion chromatogram (Herisau, Switzerland) after filtration through a 0.45 μm polypropylene filters (VWR International, Radnor, Pennsylvania). Anions were measured from single injections using a nine-point calibration curve from 0.1 – 50 mg L^{-1} ($r^2 > 0.99$). Cations were measured from single injections using a five-point calibration curve from 0.1 – 10 mg L^{-1} ($r^2 > 0.99$).

Experimental setup. Triplicate column experiments were performed in tandem under oxic conditions in a fume hood at 23°C. Empty plexiglass columns (Kimble Chase; Vineland, New Jersey) were weighed and packed with 85 mL of sediment in increments of 20 mL. After every addition of 20 mL, the columns were sonicated for 20 seconds and tamped. A layer of glass wool (Supelco, Bellefonte, Pennsylvania) and 3 mm diameter glass beads (Propper Manufacturing Co, Long Island City, New York) (furnaced at 550°C for 3 hours) were placed at the top and bottom of the column to ensure even flow distribution. The dry weight of the column packed with sediment were recorded and CO_2 (AirGas; Radnor, Pennsylvania) was upflowed for 2 minutes at 5 psi backpressure. The columns were saturated for 12 hours with the reference site groundwater at a nominal flowrate of $0.144 \text{ mL min}^{-1}$, chosen to match the Darcy velocity of the field site (0.42 m day^{-1})⁵, using an IPC high precision multichannel peristaltic pump (Ismatec, Wertheim, Germany). All tubing was 1.524 mm inner diameter Tygon® E-LFL polyvinyl chloride (PVC) (MasterFlex, Radnor, Pennsylvania). The saturated column weights were recorded, and the bulk densities and porosities (

Table 2) were determined.

Table 2. Column physical and hydrodynamic properties.

	Column 1	Column 2	Column 3
Porosity	0.36	0.35	0.37
Bulk density [g cm^{-3}]	1.66	1.63	1.62
Darcy velocity [m day^{-1}]	0.42 ± 0.01	0.42 ± 0.01	0.42 ± 0.01

The hydrodynamics of the columns were tested using bromide as a conservative tracer. A subsample of groundwater from the reference site was spiked with 3.7 mg L^{-1} bromide as potassium bromide (ACS reagent grade, Sigma-Aldrich, St. Louis, Missouri) and upflowed through the columns for 1000 minutes (equivalent to 3.9 pore volumes for columns 1 and 3 and 4 pore volumes for column 2). Bromide was eluted at the start of the experiment with bromide-free groundwater from the fire training area. Groundwater samples analyzed for bromide were filtered through $0.45 \mu\text{m}$ polypropylene filters prior to IC analysis. Concentrations of bromide were determined from single injections using a 9-point calibration curve from 0.1 to 50 mg L^{-1} ($r^2 > 0.99$). Breakthrough of bromide was determined as the pore volume at which the effluent concentration was 50% of the influent concentration and was 0.99 for column 1, 1.01 for column 2, and 0.98 for column 3 (**Figure 3**). The mass recovery of bromide was 101% for column 1, 101% for column 2, and 100% for column 3.

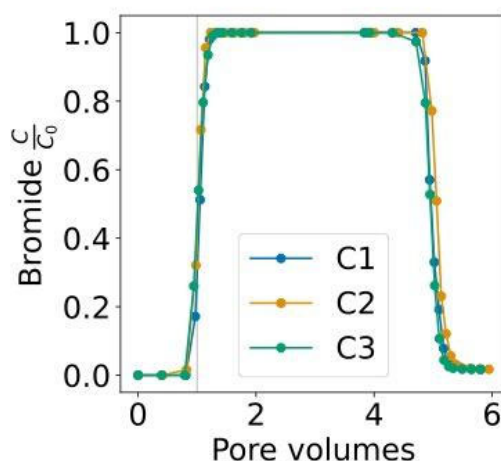


Figure 3. Bromide breakthrough curves.

The vertical gray line indicates one pore volume.

Contaminated groundwater from the fire training area was upflowed for 5000 minutes (equivalent to 19.1 pore volumes for columns 1 and 3 and 19.6 pore volumes for column 2). The columns were then eluted with groundwater from the reference site for an additional 5040 minutes (equivalent to 19.4 pore volumes for columns 1 and 3, and 19.7 pore volumes for column 2). Samples were collected throughout the experiment in 20-minute intervals in 15 mL polypropylene centrifuge tubes (Corning, Corning, New York) and stored in the refrigerator prior to PFAS analysis. The actual Darcy velocity (**Figure 4**) was determined at each sample collection point as the volume flux, determined gravimetrically, divided by the column cross sectional area. Darcy velocities were 0.42 ± 0.01 for all columns (exactly matching the field Darcy velocity) with minor fluctuations in velocity less than 2.8% for column 1, 3.8% for column 2, and 8.2% for column 3.

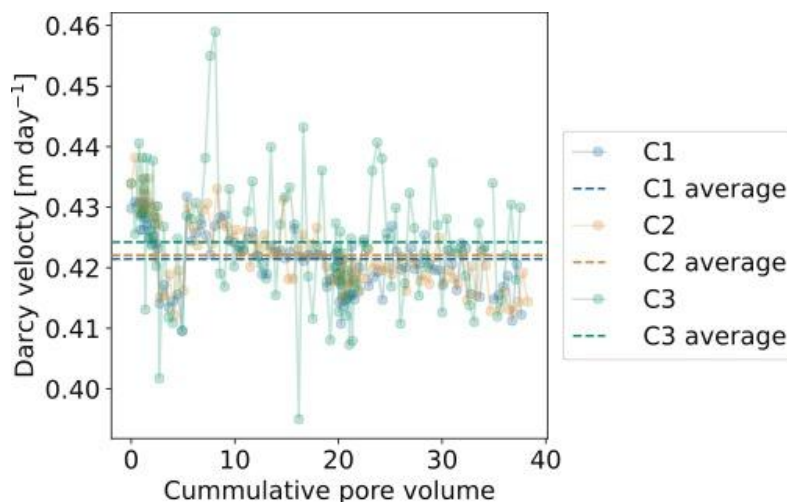


Figure 4. Column Darcy velocity over duration of the experiment.

PFAS analysis. Samples were analyzed for 17 targeted PFAS using a 6460 triple quad liquid chromatography tandem mass spectrometer (LCMS) (Agilent, Santa Clara, California) following previously published methods (Ruyle et al., 2021b). The 17 PFAS, which include the PFCAs with three through seven perfluorinated carbons (C3 – C7), the C4 –C8 PFSA, the 6:2 and 8:2 fluorotelomer sulfonates (FTSA), and the C4, C6, and C8 perfluoroalkyl sulfonamides (FSA), were chosen because they were the only compounds detected above MDL in the groundwater from the fire training area. Linear and branched isomers of PFHxS (C6) and PFOS (C8) were quantified separately using their respective native standards. Longer chained perfluoroalkyl acids, 4:2 fluorotelomer sulfonate, and other precursors with native standards available from Wellington Laboratories (Guelph, Ontario) (including zwitterionic precursors) were not detected in the groundwater.

Briefly, 750 μL aliquots were combined with 710 μL of LCMS-grade methanol and 40 μL of 0.03 $\text{ng } \mu\text{L}^{-1}$ mass-labeled internal standards (Wellington Laboratories) (**Table 3**). Concentrations of PFAS were determined from 10-point calibration curves from 1.05 to 4000 $\text{ng } \text{L}^{-1}$ ($r^2 > 0.99$). Quality control was performed by injecting a calibration point every 12 samples and the percent recovery for all PFAS was between 70 – 130%. Limits of detection were determined follow previous methods (Ruyle et al., 2021b) as the average concentration at which the sample signal-to-noise ratio was three. Method detection limits were equal to two-times the limit of detection and ranged from 2 – 25 $\text{ng } \text{L}^{-1}$. Only data from samples above the method detection limit with internal standard peak responses and qualifier ratios within 70 – 130% of the average of the calibration points were used in subsequent analyses.

Table 3. PFAS analytes in column experiments.

Analyte	Type	Internal Standard	Precursor Ion	Quantifier Ion	Quantifier Collision Energy (V)	Qualifier Ion	Qualifier Collision Energy (V)	Fragmentor Voltage (V)
PFBA	Target	[¹³ C ₄] PFBA	213.0	168.9	2			60
PFPeA	Target	[¹³ C ₅] PFPeA	262.9	218.9	2			60
PFHxA	Target	[¹³ C ₅] PFHxA	312.9	268.9	2	118.9	14	70
PFHpA	Target	[¹³ C ₄] PFHpA	362.9	318.9	2	168.9/118.9	10/18	70
PFOA	Target	[¹³ C ₈] PFOA	412.9	368.9	2	168.9	10	80
PFBS	Target	[¹³ C ₃] PFBS	298.9	80.0	38	98.9	30	95
PFPeS	Target	[¹³ C ₃] PFHxS	348.9	80.0	38	98.9	30	140
PFHxS	Target	[¹³ C ₃] PFHxS	398.9	80.0	58	98.9	34	135
PFHpS	Target	[¹³ C ₈] PFOS	448.9	80.0	54	98.9	42	180
PFOS	Target	[¹³ C ₈] PFOS	498.9	80.0	60	98.9	50	200
6:2 FTSA	Target	[¹³ C ₂] 6:2 FTSA	426.9	406.9	18	81.0	34	135
8:2 FTSA	Target	[¹³ C ₂] 8:2 FTSA	526.9	506.9	26	81.0	42	180
FBSA	Target	[¹³ C ₈] FOSA	298.0	78.0	20			140
FHxSA	Target	[¹³ C ₈] FOSA	398.0	78.0	40			180
FOSA	Target	[¹³ C ₈] FOSA	497.9	78.0	38			140
[¹³ C ₄] PFBA	ISTD		216.9	171.9	2			60
[¹³ C ₅] PFPeA	ISTD		267.9	223.0	2			60
[¹³ C ₅] PFHxA	ISTD		317.8	273.0	2			70
[¹³ C ₄] PFHpA	ISTD		366.8	321.9	2			70
[¹³ C ₈] PFOA	ISTD		420.9	376.0	2			75
[¹³ C ₃] PFBS	ISTD		301.9	99.0	26			95
[¹³ C ₃] PFHxS	ISTD		401.9	98.9	38			180
[¹³ C ₈] PFOS	ISTD		506.9	99.0	50			180
[¹³ C ₂] 6:2 FTSA	ISTD		428.9	81.0	46			95
[¹³ C ₂] 8:2 FTSA	ISTD		528.9	81.0	46			180
[¹³ C ₈] FOSA	ISTD		505.9	78.0	38			95

Determining sediment-water partitioning coefficients. Sediment-water partitioning coefficients were determined using EPA Guidelines (the breakthrough method, Krupka et al., 1999) and Hydrus-1D (version 4.17.0140, Šimůnek et al., 2016). K_d values estimated from the breakthrough method were calculated using Equations 7-9:

$$K_{d,x} = \frac{(R_x - 1) \eta}{\rho_b} \quad (\text{Equation 7})$$

$$R_x = \frac{v_{Br^-}}{v_x} \quad (\text{Equation 8})$$

$$v_i = \frac{h_{column}}{t_{i,breakthrough}} \quad (\text{Equation 9})$$

where, $K_{d,x}$ is the sediment-water partitioning coefficient for solute x ($L \text{ kg}^{-1}$), η is the porosity, ρ_b is the bulk density (kg L^{-1}), v_{Br^-} is the velocity of the conservative bromide tracer, v_x is the velocity of compound x, h_{column} is the height of column, and $t_{i,breakthrough}$ is the time where the effluent concentration is half of the influent concentration of compound i.

Values for K_d for compounds with retardation coefficients greater than 1.15 were estimated using Hydrus-1D by fitting the advection-dispersion equation to the observations (liquid-phase concentrations from column effluent). For all compounds, the single porosity van Genuchten – Maulem model with no hysteresis was used with a saturated soil water content (θ_s) equal to the porosity and a saturated hydraulic conductivity (K_s) equal to 0.1 cm min^{-1} . Constant water (0.42 m day^{-1}) and solute concentration (

Table 1) flux boundary conditions at the upper boundary and free drainage and zero solute concentration gradient boundary conditions at the lower boundary were imposed. An equilibrium solute transport model was used for all compounds except for the perfluoroalkyl sulfonamides. Initial estimates of K_d were set equal to those estimated from the breakthrough method. A single optimal K_d for compounds which did not exhibit a concentration spike during elution (PFHpA, Linear PFHxS, Branched PFHxS, FBSA, and FHxSA) was determined using data from both loading and eluting phases. Two values for K_d , one for the loading phasing and another for the eluting phase, were determined for compounds that exhibited a concentration spike during elution (PFOA, PFHpS, Linear PFOS, Branched PFOS, 6:2 FTSA, and 8:2 FTSA).

Mass recovery was calculated as the total measured mass divided by the input mass. The total measured mass was estimated as the Riemann sum of the product of the measured solute concentration and volumetric flowrate multiplied by the change in time between each sampling point. The input mass was calculated as the solute concentration in the fire training area groundwater multiplied by the volume of groundwater pumped through the column. Complete mass recovery within $\pm 10\%$ was observed for all compounds except FHxSA ($42 \pm 18\%$) and FOSA (which was not measured at any sampling point during the experiment) (**Table 4**).

Table 4. Column experiment PFAS mass recovery.

PFAS	Column 1	Column 2	Column 3	Column average $\pm$ standard deviation
PFBA	0.99	1.00	1.03	1.01 ± 0.02
PFPeA	1.02	1.03	1.04	1.03 ± 0.01
PFHxA	1.02	1.01	1.01	1.01 ± 0.00
PFHpA	1.07	1.06	1.04	1.06 ± 0.01
PFOA	1.02	1.03	1.00	1.02 ± 0.01
PFBS	1.01	0.98	0.96	0.98 ± 0.02
PFPeS	1.01	1.00	1.00	1.00 ± 0.00
Linear PFHxS	1.03	1.05	0.99	1.02 ± 0.02
Branched PFHxS	1.01	1.00	0.99	1.00 ± 0.01
PFHpS	0.98	0.96	0.92	0.96 ± 0.02
Linear PFOS	1.11	1.09	0.92	1.04 ± 0.09
Branched PFOS	1.02	1.06	0.96	1.01 ± 0.04
62 FTSA	0.93	1.02	1.02	0.99 ± 0.04
82 FTSA	1.40	1.07	0.74	1.07 ± 0.27
FBSA	0.94	1.00	0.85	0.93 ± 0.06
FHxSA	0.65	0.37	0.23	0.42 ± 0.18
FOSA	0.00	0.00	0.00	0.00 ± 0.00

Task 2 Methods: Precursor biotransformation

Materials. N-(3-dimethylaminopropan-1-yl)-perfluoro-1-hexane-sulfonamide (PFHxSAm, **Table 5**) was supplied by Wellington Labs (N-AP-FHxSA, 50 µg/mL in methanol). The AFFF formulation used for spiking experiments was a sulfonamide-based 3M Light Water™ (3% Concentrate, 2001), which has been characterized and quantified by HPLC-MS/MS (

Table 6). To avoid growth of C1 metabolizing organisms in the microbial experiments, PFHxSAm standards were evaporated to dryness to remove methanol (MeOH) under a gentle stream of nitrogen and reconstituted with DI water to achieve the desired spiking concentration. Diethylene glycol butyl ether (DGBE) was purchased from Sigma Aldrich. Groundwater, surface water, and sediment were collected from various local sources such as Ashumet Pond, MA, near the JBCC in August 2020 using pre-washed 1 L polypropylene bottles.

Table 5. Key PFAS analyzed in biodegradation experiments.

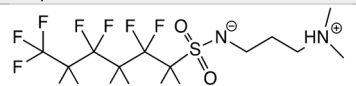
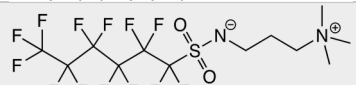
Abbreviation	Chemical name	Structure
PFOS	Perfluorooctanesulfonate	
PFHxS	Perfluorohexanesulfonate	
FHxSA	Perfluorohexanesulfonamide	
PFHxSAm (N-AP-FHxSA)	N-(3-dimethylaminopropan-1-yl)- perfluoro-1-hexane-sulfonamide	
PFHxSAmS (N-TAmP-FHxSA)	N-[2-(perfluoro-1-hexanesulfonamido)- propan-1-yl]-N,N,N-trimethylammonium	

Table 6. Concentrations of PFAS and other organics in 3M Light Water™ 3% (2001).

Compound	Concentration
<i>PFAS</i>	<i>[mg/L]</i>
Linear PFOS	10412
Branched PFOS	3333
Linear PFHxS	1357
Branched PFHxS	410
FHxSA	11.6
PFHxA	364
PFHxSA _m	9714
PFHxSA _m S	451
<i>Organic components</i>	<i>[%]</i>
Diethylene glycol butyl ether	20
Alkyl sulfate salts	1-5
Triethanolamine	0.5-1.5
Tolyl triazole	0.05

Biotransformation Experiments.

Biotransformation microcosms were set up to replicate microbial association and transformation conditions at the groundwater/surface water boundary (Figure 5). Microcosms were constructed using a slurry of discharging groundwater and lake bottom sediment collected from Ashumet Pond, Massachusetts, United States that was stored at 4°C prior to use. Ashumet Pond is a mesotrophic kettle lake that receives PFAS inputs from an upgradient former fire-training area at Joint Base Cape Cod (JBCC) (Tokranov et al. 2021, Weber et al. 2017). Concentrations of targeted PFAS analytes in this study were below method detection limits in the source water. Solids consist of primarily sand and gravel minerals and have an organic carbon content of <0.1% (Weber et al. 2017).

Design of the biotransformation microcosms followed previously published experiments with slight modifications (Harding-Marjanovic et al. 2015). For all experiments, triplicate microcosms containing 30 mL of water and 10 g sediment were set up in 158-mL septum-capped clear glass serum bottles. Abiotic controls, consisting of either autoclaved Ashumet Pond water and sediment or sterile Milli-Q water without sediment, were incubated with the biotic experiments. Sodium azide (0.5 g L⁻¹) was added to the control microcosms to ensure abiotic conditions were maintained throughout the duration of the experiment. Individual stocks of PFHxSA_mS, PFHxSA_m, and FHxSA (Wellington Laboratories, Guelph, Ontario, Canada), were blown down under a gentle stream of nitrogen to dryness and reconstituted in sterile Milli-Q water to avoid enhanced microbial metabolism using methanol. The reconstituted stocks were spiked into the bottles for an initial concentration between 14 and 95 nanomolar (nM).

The microcosms were incubated in the dark at 19°C or 29°C with shaking (150 rpm). Experiments ran for 45 (29°C conditions) or 60 (19°C conditions) days and were sampled 13

times each. The pH was monitored throughout the experiments and was typically between 6-7.2. At each sampling timepoint, an aliquot of 1.3-2.3 mL was collected using a 3 mL syringe fitted with a 22G needle. Filtered air was injected after sampling to maintain aerobic conditions. Aliquots were centrifuged for 15 min at 13,000 rpm to remove sediment particles and cells, and aqueous samples were split for PFAS and nitrate analyses. Samples for PFAS determination were stored at -20°C prior to analysis within a month.

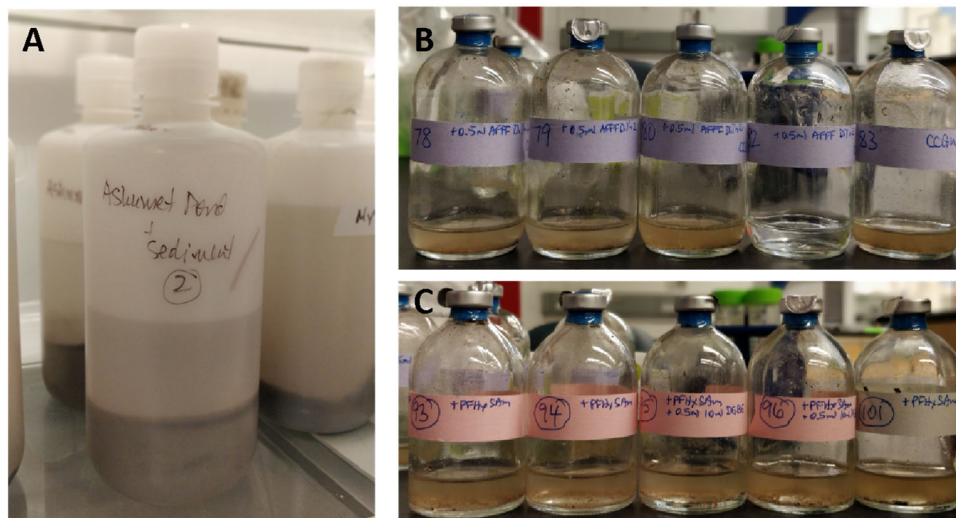


Figure 5. Biotransformation experimental setup.

Images of A) sediment-water mixture from Ashumet Pond and B) Experiment A (AFFF) and C) Experiment B (PFHxSAm). Pictures B and C are taken at day 53 and 47, respectively.

Slurry sampling. At each time point, slurry aliquots (1.3-2.3 mL) were sampled with a 3 mL syringe fitted with a 22G needle for targeted PFAS LC-MS/MS and TOC analysis. Samples were centrifuged (15 min at 12000 rpm) to remove sediment particles and cells. After centrifugation, 1.0 mL of supernatant was removed for TOC analysis and diluted 1:6 or 1:8 times (depending on expected concentration) with DI water. Then, 100 μ L of supernatant was diluted with 650 μ L DI water, 710 μ L MeOH, and stored at -20°C until LC-MS/MS analysis. On the day of analysis, samples were thawed at room temperature, spiked with 40 μ L of the PFAS internal standard mixture, and analyzed by HPLC-MS/MS. Samples for DNA analysis (0.5 mL) were taken at t_0 and t_{final} , and stored at -80°C. Samples for cell enumeration (0.5 mL amended with 20 μ L glutaraldehyde) were taken at 4-5 intermittent time points during the course of the experiment that included t_0 and t_{final} , and stored at -80°C. Experiments A and B were monitored over 32 and 26 days, respectively. Sampling took place approximately twice a week.

HPLC-MS/MS precursor method development. Several studies have demonstrated the presence of zwitterionic PFAS species in sulfonamide based AFFF formulations (Houtz et al., 2013). Therefore, LC-MS/MS method development was carried out to include two novel precursor zwitterions (PFHxSAm and PFHxSAmS, **Table 5**) into the existing analysis method of 29 PFAS (including PFHxS and FHxSA). As PFHxSAm and PFHxSAmS typically show higher sensitivity in positive mode ESI(+) as compared to negative mode ESI(-) (in which all other compounds are analyzed), rapid polarity switching was applied to combine analysis of all

compounds into one method. The chromatographs of the two primary transitions for both zwitterions are displayed in **Figure 6**. The presence of PFHxSAm branched isomer(s) is suggested due to a smaller peak eluting slightly earlier than the major linear isomer peak. Due to the lower MS signal of PFHxSAmS compared to PFHxSAm (note the order of magnitude difference in y-axis), the branched isomers were only visible for the latter compound. As only native standards and no isotopically-labeled internal standards are commercially available, the concentration of these compounds reported here should be considered semi-quantitative.

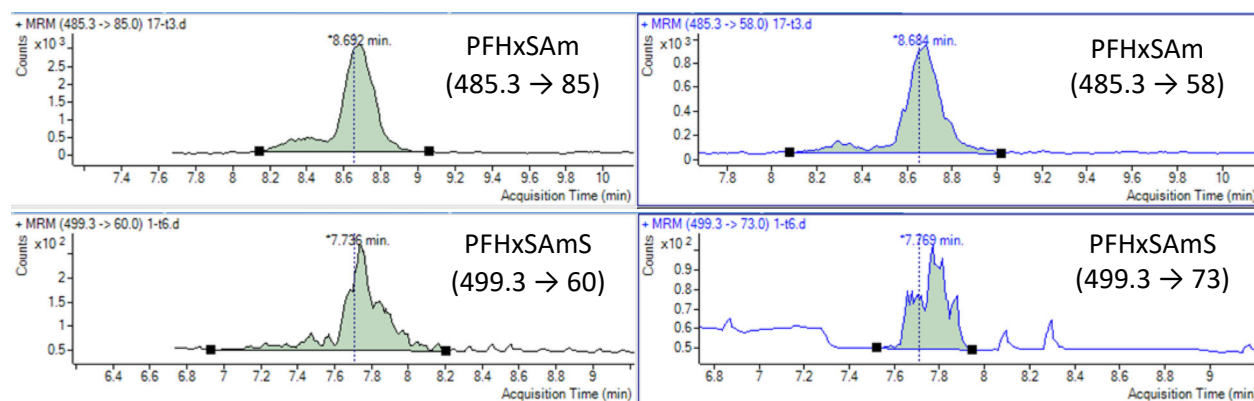


Figure 6. LC-MS/MS chromatographs of PFHxSAm and PFHxSAmS.

Top row shows PFHxSAm (transitions 485.3 → 85 and 485.3 → 58) with visible branched isomers eluting before main linear isomer; and bottom row shows PFHxSAmS (transitions 499.3 → 60 and 499.3 → 73) in AFFF-spiked slurries.

Suspect screening. Suspect screening analysis was performed using a SCIEX ExionLC AC UHPLC system coupled to a SCIEX X500R quadrupole time-of-flight tandem mass spectrometer (QTOF MSMS). A Phenomenex Gemini 3um C18 110Å 50x2mm LC analytical column preceded with a Phenomenex SecurityGuard cartridge was used for the analyte separation. Another Phenomenex Gemini 5um C18 110 Å 50x4.6mm LC analytical column was used to delay the PFAS instrumental contribution. The aqueous mobile phase (MPA) was 10mM ammonium acetate in water and the organic mobile phase (MPB) was 10mM ammonium acetate in methanol. LC parameters were set as follows: flow 0.3mL/min, injection 20uL, column oven 45°C. Solvent gradient of MPB gradually increased from 40% to 80% (1 to 5.5 min), 80% to 100% (5.5 to 7 min), held for one minute and finally drop to 40% (8 to 8.5 min) and held for another 6.5 minutes.

The MS data for the suspect screening analysis were collected using both IDA and SWATH acquisitions. ESI operated in negative and positive mode with the following parameters was used: curtain Gas at 30 psi, ion source gas 1 at 40 psi, ion source gas 2 at 60 psi, temperature 450°C. The compounds were tentatively identified using the suspect screening approach, based on precursor mass, isotope pattern, retention time, exact mass accuracy (<5ppm), and MS/MS fragmentation matching (SCIEX Fluorochemical HR-MS/MS Spectral Library 2.0) (Table 7). Following the conventions from Schymanski et al. (2014), both compounds were assigned the confidence level of identification 2a (MS/MS spectral library match).

Table 7. Exact masses of potential precursor metabolites.

Analyte	Exact mass [Da]
PFHxSAmS	499.0719
PFHxSAm	484.0490
Secondary amine	470.0334
Primary amine	456.0177
Aldehyde	454.9861
Propanoic acid	479.9810
Acetic acid (FHxSAA)	456.9653
FHxSA	398.9599
PFHxSi	383.9490
PFHxS	399.9439

Modeled Rates and Biotransformation Box model.

Timeseries concentration data from the biotransformation microcosms were modeled using equations based on Michaelis-Menten kinetics for enzyme-mediated reactions (Schwarzenbach et al., 2017). Model parameters to account for sorption to the glassware/sediment were included (Plazinski et al., 2009). Equations were fit to data from the triplicate microcosms using Markov-chain Monte Carlo (MCMC) analysis to quantify uncertainty (Equations 10, 11) (Gelman et al., 2004). MCMC analysis was performed using Python *emcee* version 3.1.1 (Foreman-Mackey et al., 2013) to estimate the expected mean and 90th percentile confidence interval (CI) for the rates.

Kinetic rates (k) were fit to the experimental data (X) using MCMC analysis (Equation 10):

$$P(k|X) \propto P(X|k) \times P(k) \quad (\text{Equation 10})$$

Where $P(k|X)$ is the posterior probability distribution of the data-optimized kinetic rates, $P(X|k)$ is the likelihood equation, and $P(k)$ is the prior. For each rate (k), we implemented normal priors parameterized by the mean and standard deviation of a non-linear least squares fitting of the experimental data implemented in *scipy* version 1.7.3.¹⁶ The likelihood was the squared error between the measurements and the modeled concentration shown in Equation 11:

$$P(X|K) = (X - f(k))^2 \quad (\text{Equation 11})$$

Where $f(k)$ is either first-order loss of the precursor spike or zero-order production of PFHxS, depending on the data being modeled.

MCMC analysis was performed using *emcee* version 3.1.1 with four independently seeded walkers that update their sampling location according to the differential evolution Snooker algorithm. The analyses were run until the MCMC error was $2500^{-0.5}$. We present

results for the expected mean and 90th confidence interval throughout the manuscript. The code is available for use at <https://github.com/SunderlandLab/pfas-precursor-biotransformation>.

We temporally extended the experimental data to better understand the timescales required for PFHxS formation from precursor biotransformation using a four-compartment box model parameterized with the measured rate constants. Experimentally measured zero- and first-order reactions were used to develop a four-box model to better understand the temporal evolution of precursors and terminal compounds beyond the duration of the experiments performed (**Figure 7**). The sorbed (sediment/glassware) and biological reservoirs of PFHxS were neglected because they are not substantial reservoirs in our experimental setup.

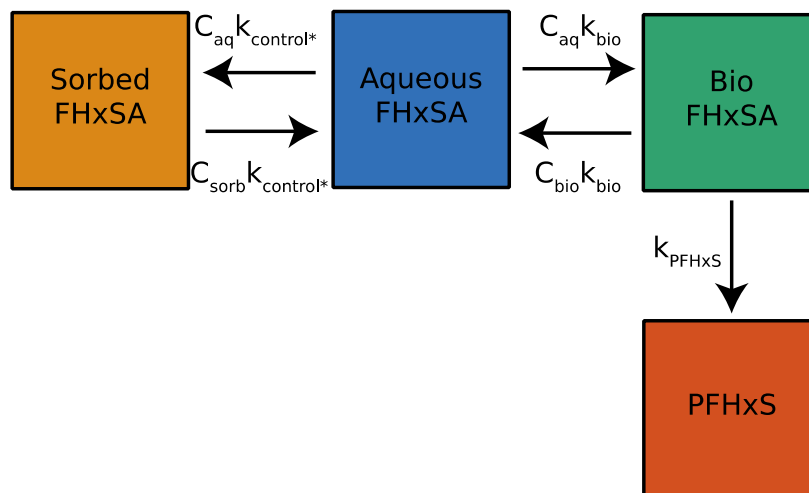


Figure 7. Biotransformation box model.

Schematic of four box model parameterized using experimentally determined rates for association/sorption of the precursors to microbes (k_{bio}), reversible sorption to the sediment/glassware ($k_{control*}$), and formation of terminal degradation product (k_{PFHxS}), and used to simulate biotransformation of precursors beyond the temporal period measured experimentally.

The PFHxS mass balance is shown in Equation 12:

$$M_{tot} = M_{aq} + M_{sorb} + M_{bio} \quad (\text{Equation 12})$$

Where M is the mass of PFHxS in various reservoirs. Substituting partitioning coefficient (K_d for sediment/water and K_{oc} for microbial/water) into Equation 12 results in Equation 13:

$$M_{tot} = c_{aq}(V + k_d M_{sed} + K_{oc} M_{bio}) \quad (\text{Equation 13})$$

Where c_{aq} is the aqueous phase concentration of PFHxS, V is the water volume (35 mL), M_{sed} is the sediment mass (10 g), and M_{bio} is the microbial mass. The term for the sorbed reservoir can be neglected because $\log K_d$ of PFHxS < -1.5 to Cape Cod sediment (Weber et al.,

2017). Using the range of $\log K_{oc}$ (0.66 – 2.0) reported in prior work at JBCC (Weber et al., 2017), M_{bio} would have to be 0.35–7.6 g for the biological reservoir to be greater than the aqueous reservoir. The biomass to sediment ratio in the microcosms would therefore have to be approximately 1:10 – 1:1. Although the mass of biomass was not directly measured, we assume that such large ratios are not likely because the fraction of organic carbon on Cape Cod sediment is <0.1% by weight (Weber et al., 2017; Barber et al., 1992).

Task 3 Methods: JBCC fire-training area model

In our go/no-go report, we proposed a larger groundwater model describing the full A-A' transect reported in Weber et al. (2017). Additional sample analyses of the plume between 2002 and 2021 showed little variability in PFAS concentrations beneath the fire training area but substantial variability in the downstream plume. From these data, we determined that even more sampling to fully define the position and extent of the contamination would be needed to create a model of the entire groundwater plume. We have therefore refined the spatial domain of the model to consider PFAS transport and transformation in the immediate vicinity of the fire training area, where extensive data is available in time from USGS archival samples and data reported by AFCEC. We have provided the temporal concentrations of PFAS we measured in the downstream plume but did not use for this project to Ms. Rose Forbes, AFCEC JBCC site-manager. To constrain the more spatially limited model, we leveraged from archived USGS groundwater samples from the fire training area between 2002 and the present as well as data reported by the AFCEC (Unpublished Technical Updates, Personal Communications, Rose Forbes, AFCEC) in groundwater and soil.

We developed an analytical and statistical toolbox based on measurements of targeted PFAA, oxidizable precursors interpreted using Bayesian inference, and extractable organofluorine (EOF) to identify PFAS originating from AFFF (Ruyle et al. 2021a). This toolbox can quantify all PFAS originating from legacy ECF AFFF produced by 3M and contemporary FT AFFF that currently are MILSPEC approved (Ruyle et al. 2021a) (**Figure 8**).

Our statistical inference procedure differentiates the relative use of ECF versus FT AFFF in the environment (Ruyle et al. 2021b) (**Figure 9**), which is particularly useful at military fire training areas where both types of products were often used but poor record keeping prevents a priori knowledge of how much of each type of AFFF was used (Place and Field, 2012). For example, if ECF AFFF (approximately 50% precursors) and FT AFFF (>99% precursors) were used in equal amounts, the percentage of ECF precursors in downstream environments would be approximately 33%, assuming that the biogeochemical influences on precursor composition affect both kinds of precursors at similar rates. We find that ECF precursors accounted for $72 \pm 11\%$ of precursors in the Quashnet River watershed downstream of the fire training area suggesting ECF AFFF was used at a greater than 2:1 ratio at the fire training area (Ruyle et al. 2021b).

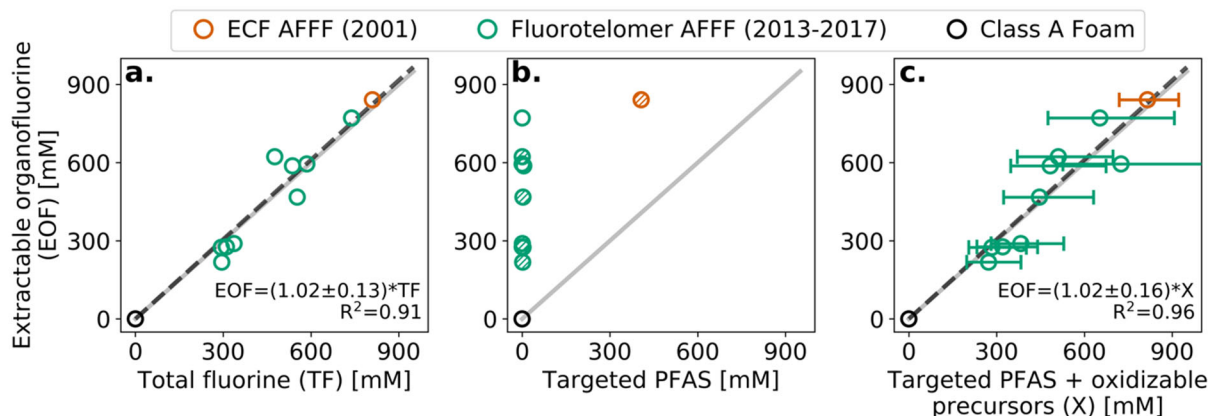


Figure 8. Fluorine measurements in AFFF performed using four independent techniques.

Panel (a) compares concentrations of extractable organofluorine (EOF) and total fluorine (TF). Panel (b) compares EOF and targeted PFAS detected using LC-MS/MS. Hatching of circles denotes AFFF containing PFAS purportedly phased out since 2015 by the PFOA Stewardship Program. Panel (c) shows EOF and the sum of targeted PFAS and polyfluoroalkyl compounds (unknown) detected using the TOP assay. Error bars represent the 25% and 75% of inferred oxidizable precursors using Markov Chain Monte Carlo (MCMC) Bayesian inference method. Ordinal least squares (a) and weighted least squares (c) linear regression of PFAS-based AFFF (black dash) in panels is compared to the 1:1 line (solid gray). Figure from Ruyle et al. (2021a).

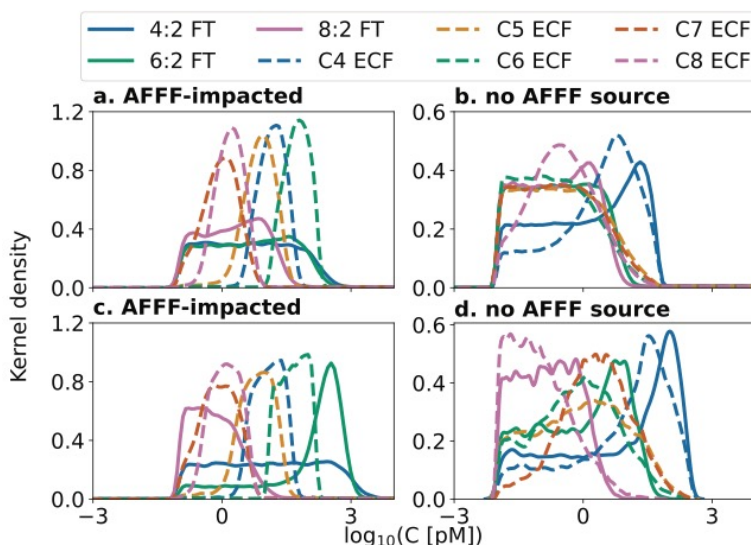


Figure 9. Inferred perfluorinated chain-length and manufacturing origins of oxidizable precursors.

Results from Bayesian inference on results of the TOP assay. Panels show probability density functions estimated by the non-parametric kernel density of the concentrations of oxidizable precursors. A high kernel density indicates greater probability of the estimate. Precursors are grouped by perfluorinated chain length and manufacturing source. Electrochemical fluorination (ECF) precursors range from 4-8

perfluorinated carbons (C4-C8) while fluorotelomer (FT) precursors have n perfluorinated carbons followed by two aliphatic hydrocarbons ($n:2$, $n=4,6,8$). Samples downstream of AFFF source zones are shown in panel (a) the Quashnet R., and panel (c) Mill Creek. Samples from background sites are shown in panel (b) the Santuit R., and (d) Marstons Mills R. Figure from Ruyle

Our modeling efforts focused on interpreting data from a USGS monitoring well MA-SDW 425-0063 (AFCEC well ID 30MW0417C) beneath the former fire training area. The well is screened 16.2-19.3 m below ground surface. We measure PFAS sampled from the well on 9/18/2007, 07/09/2014, 07/06/2015, 11/09/2015, 06/30/2020, and 07/20/2021. We also included in our analyses data reported in from this well in 06/04/2014, 07/09/2014, 11/09/2015, 09/20/2019, 06/30/2020 by Weber et al., 2017, AFCEC data (Unpublished Technical Updates, Personal Communications, Rose Forbes, AFCEC), and the National Institute of Standards and Technology.

Targeted analysis. Samples were analyzed for up to 33 PFAS (**Table 8**) including the C3-C13 PFCA, C4-C10 PFSA (the linear and branched isomers of PFHxS and PFOS were quantified individually from their respective standards), the $n:2$ ($n = 4, 6, 8, 10$) fluorotelomer sulfonates, 6:2 fluorotelomer sulfonamido betaine, the C_n ($n = 4, 6, 8, 10$) FASA, the C8 perfluoroalkyl sulfoamido acetates, and PFHxSAm and PFHxSAmS. Sample analysis was performed on Agilent (Santa Clara, California, United States) 6460 triple quadrupole liquid chromatography-tandem mass spectrometer at Harvard University following the procedure in prior work (**Table 8**) (Weber et al. 2017; Ruyle et al. 2021b). Prior to subsampling, sample bottles were removed from the refrigerator, warmed to room temperature, shaken vigorously, and sonicated three times for twenty second. An aliquot of 750 mL was combined with 710 mL of LCMS-grade methanol (Honeywell, Charlotte, North Carolina, United States) and 40 mL of 0.03 ng mL⁻¹ mass-labeled internal standard mixture (Wellington Laboratories; Guelph, Ontario, Canada; Table S6) in a polypropylene microcentrifuge tube (Sorenson BioScience Inc., Murray, Utah, United States). The samples were vortexed and centrifuged. A 900 mL aliquot was transferred to a 1000 mL polypropylene vial (Agilent) for instrumental analysis. All analytes were monitored for in electrospray ionization (ESI) negative mode except for 6:2 FTAB, PFHxSAm, and PFHxSAmS which were monitored for in ESI positive mode.

Table 8. LC-MS/MS parameter for PFAS measurements supporting Task 4.

Analyte	Type	Internal Standard	Precursor Ion	Quantifier Ion	Quantifier Collision Energy (V)	Qualifier Ion	Qualifier Collision Energy (V)	Fragmentor Voltage (V)
PFBA	Target	[¹³ C ₄] PFBA	213.0	168.9	2			60
PFPeA	Target	[¹³ C ₅] PFPeA	262.9	218.9	2			60
PFHxA	Target	[¹³ C ₅] PFHxA	312.9	268.9	2	118.9	14	70
PFHpA	Target	[¹³ C ₄] PFHpA	362.9	318.9	2	168.9/118.9	10/18	70
PFOA	Target	[¹³ C ₈] PFOA	412.9	368.9	2	168.9	10	80
PFNA	Target	[¹³ C ₉] PFNA	462.9	418.9	2	218.9/169.0	10/14	75
PFDA	Target	[¹³ C ₆] PFDA	512.9	468.9	6	269.0/218.9	14/14	85
PFUnDA	Target	[¹³ C ₇] PFUnDA	562.9	518.9	6	269.0/169.0	14/22	95
PFDoDA	Target	[¹³ C ₂] PFDoDA	612.9	569.0	6	269.0/169.0	14/26	90
PFTeDA	Target	[¹³ C ₂] PFTeDA	662.8	618.9	6	169.0	26	95
PFTeDA	Target	[¹³ C ₂] PFTeDA	712.9	669.0	6	169.0	25	100
PFBS	Target	[¹³ C ₃] PFBS	298.9	80.0	38	98.9	30	95
PFPeS	Target	[¹³ C ₃] PFHxS	348.9	80.0	38	98.9	30	140
PFHxS	Target	[¹³ C ₃] PFHxS	398.9	80.0	58	98.9	34	135
PFHpS	Target	[¹³ C ₈] PFOS	448.9	80.0	54	98.9	42	180
PFOS	Target	[¹³ C ₈] PFOS	498.9	80.0	60	98.9	50	200
PFNS	Target	[¹³ C ₈] PFOS	548.9	80.0	60	98.9	54	175
PFDS	Target	[¹³ C ₈] PFOS	598.9	80.0	60	98.9	54	175
4:2 FTSA	Target	[¹³ C ₂] 4:2 FTSA	326.9	307.0	10	81.0	30	130
6:2 FTSA	Target	[¹³ C ₂] 6:2 FTSA	426.9	406.9	18	81.0	34	135
8:2 FTSA	Target	[¹³ C ₂] 8:2 FTSA	526.9	506.9	26	81.0	42	180
10:2 FTSA	Target	[¹³ C ₂] 8:2 FTSA	637.0	607.0	30	81.0	70	180
6:2 FTAB	Target	[¹³ C ₂] 6:2 FTSA	571.4	440.0	20	104.0/58.0	35/35	190
FBSA	Target	[¹³ C ₈] FOSA	298.0	78.0	20			140
FHxSA	Target	[¹³ C ₈] FOSA	398.0	78.0	40			180
FOSA	Target	[¹³ C ₈] FOSA	497.9	78.0	38			140
FDSA	Target	[¹³ C ₈] FOSA	598.0	78.0	32			140
N-EtFOSAA	Target	d5-N-EtFOSAA	583.9	418.9	18	525.9	14	95
N-MeFOSAA	Target	d3-N-MeFOSAA	569.9	418.9	14	482.9	10	95
PFHxSAm	Target	[¹³ C ₃] PFHxS	485.3	85.0	30	58.0	35	175
PFHxSAmS	Target	[¹³ C ₃] PFHxS	499.3	73.0	35	60.0	35	170
[¹³ C ₄] PFBA	ISTD		216.9	171.9	2			60
[¹³ C ₅] PFPeA	ISTD		267.9	223.0	2			60
[¹³ C ₅] PFHxA	ISTD		317.8	273.0	2			70
[¹³ C ₄] PFHpA	ISTD		366.8	321.9	2			70

[¹³ C ₈] PFOA	ISTD	420.9	376.0	2	75
[¹³ C ₉] PFNA	ISTD	472.0	427.0	2	85
[¹³ C ₆] PFDA	ISTD	518.9	474.0	2	90
[¹³ C ₇] PFUnDA	ISTD	569.9	525.0	6	85
[¹³ C ₂] PFD _o DA	ISTD	614.9	569.9	6	95
[¹³ C ₂] PFTeDA	ISTD	714.8	670.0	6	95
[¹³ C ₃] PFBS	ISTD	301.9	99.0	26	95
[¹³ C ₃] PFHxS	ISTD	401.9	98.9	38	180
[¹³ C ₈] PFOS	ISTD	506.9	99.0	50	180
[¹³ C ₂] 4:2 FTSA	ISTD	328.9	81.0	38	95
[¹³ C ₂] 6:2 FTSA	ISTD	428.9	81.0	46	95
[¹³ C ₂] 8:2 FTSA	ISTD	528.9	81.0	46	180
[¹³ C ₈] FOSA	ISTD	505.9	78.0	38	95
d5-N-EtFOSAA	ISTD	588.9	418.9	14	95
d3-N-MeFOSAA	ISTD	572.9	418.9	14	100

PFAS concentrations were determined from a 12-point calibration curve ranging from 1 to 20,000 ng L⁻¹ (**Table 9**). The linear and branched isomers of PFHxS and PFOS were quantified separately using calibration curves for each isomer. We refer to PFOS and PFHxS throughout the report as the sum of isomers to be comparable with data from other sources that did not distinguish them (Weber et al. 2017; Unpublished Technical Updates, Personal Communications, Rose Forbes, AFCEC). Method detection limits (MDL) ranged from 2.1 to 40 ng L⁻¹ (<10x the minimum concentrations of PFAS at the focus of this study) and were calculated as three times the signal to noise of the samples with qualifier/quantifier ratios $\pm 30\%$ of the 4000 ng L⁻¹ calibration (**Table 10**). Concentrations of PFAS were always <MDL in two instrumental Milli-Q water blanks. PFAS were <MDL in a nearby reference well thought to be PFAS free sampled using the same equipment and procedure contemporaneously to data reported here for 2007, 2020, and 2021. Duplicate injections were performed every five samples and the average percent difference for all analytes was < 20% except for 6:2 FTSA (39%) and FOSA (27%). We do not use the concentrations of individual precursors such as 6:2 FTSA or FOSA in analyses in this work. Internal standard peak areas for all samples and analytes were always within $\pm 30\%$ of the average of the calibration points.

Table 9. Targeted PFAS Concentrations in Groundwater [nM].

PFAS	09/18/07	06/04/14 ¹	07/09/14	07/06/15 ¹	11/09/15	06/30/20	07/20/21
PFBA	5.0	1.3	1.0	2.3	1.4	3.4	0.7
PFPeA	13.2	2.7	2.2	5.2	3.3	8.6	1.3
PFHxA	24.0	4.0	3.2	9.5	6.7	16.1	1.8
PFHpA	7.6	1.6	1.3	5.4	3.3	5.0	0.4
PFOA	35.5	5.8	4.9	17.1	17.7	56.9	1.7
PFNA	0.1	<MDL	<MDL	<MDL	0.1	0.0	<MDL
PFDA	NR	<MDL	<MDL	<MDL	<MDL	NR	<MDL
PFUnDA	NR	<MDL	<MDL	<MDL	<MDL	NR	<MDL
PFDODA	NR	<MDL	<MDL	<MDL	<MDL	NR	<MDL
PFTTrDA	NR	NR	<MDL	NR	<MDL	NR	<MDL
PFTeDA	NR	NR	<MDL	NR	<MDL	NR	<MDL
PFBS	2.5	0.9	0.5	1.4	0.8	1.9	0.2
PFPeS	NR	0.0	1.0	NR	3.7	NR	0.3
Linear PFHxS	59.0	0.0	13.2	NR	43.6	64.0	4.2
Branched PFHxS	9.5	0.0	2.3	NR	7.3	7.5	0.2
PFHxS	68.5	24.0	15.5	38.6	51.0	71.5	4.4
PFHpS	NR	0.0	0.4	NR	1.0	NR	0.0
Linear PFOS	30.9	0.0	7.2	NR	9.0	11.3	3.1
Branched PFOS	13.5	0.0	3.4	NR	4.7	5.7	0.3
PFOS	44.4	12.9	10.6	10.0	13.7	17.0	3.4
PFNS	NR	<MDL	NR	NR	<MDL	NR	<MDL
PFDS	NR	0.0	<MDL	<MDL	<MDL	NR	<MDL
4:2 FTSA	NR	NR	<MDL	NR	<MDL	NR	<MDL
6:2 FTSA	7.1	NR	2.1	6.6	6.0	23.6	0.5
8:2 FTSA	1.3	NR	0.8	1.2	0.5	0.9	0.7
10:2 FTSA	NR	NR	<MDL	NR	<MDL	NR	<MDL
6:2 FTAB	NR	NR	<MDL	NR	<MDL	NR	<MDL
FBSA	4.3	NR	0.5	NR	1.0	2.4	0.3
FHxSA	10.9	NR	2.4	NR	2.8	3.6	1.5
FOSA	0.1	NR	0.2	0.3	0.1	0.3	0.2
FDSA	NR	NR	<MDL	NR	<MDL	NR	<MDL
PFHxSAm	<MDL	NR	<MDL	NR	<MDL	NR	<MDL
PFHxSAmS	<MDL	NR	<MDL	NR	<MDL	NR	<MDL

¹Targeted PFAS data originally reported in Weber et al. (2017)

NR = not reported

<MDL = less than method detection limit

Table 10. PFAS Method Detection Limits [nM].

PFAS	09/18/07	06/04/14 ¹	07/09/14	07/06/15 ¹	11/09/15	06/30/20	07/20/21
PFBA	0.044	0.087	0.053	0.092	0.053	0.037	0.053
PFPeA	0.044	0.043	0.055	0.157	0.055	0.031	0.055
PFHxA	0.023	0.031	0.035	0.028	0.035	0.026	0.035
PFHpA	0.029	0.021	0.006	0.015	0.006	0.036	0.006
PFOA	0.026	0.020	0.022	0.024	0.022	0.026	0.022
PFNA	0.044	0.025	0.005	0.016	0.005	0.040	0.005
PFDA	NR	0.046	0.004	0.023	0.004	NR	0.004
PFUnDA	NR	0.013	0.003	0.002	0.003	NR	0.003
PFDODA	NR	0.012	0.001	0.002	0.001	NR	0.001
PFTTrDA	NR	NR	0.001	NR	0.001	NR	0.001
PFTeDA	NR	NR	0.049	NR	0.049	NR	0.049
PFBS	0.018	0.044	0.013	0.018	0.013	0.017	0.013
PFPeS	NR	NR	0.017	NR	0.017	NR	0.017
Linear PFHxS	0.016	NR	0.033	NR	0.033	0.019	0.033
Branched PFHxS	0.014	NR	0.038	NR	0.038	0.019	0.038
PFHxS	NA	0.013	NA	0.014	NA	NA	NA
PFHpS	NR	NR	0.031	NR	0.031	NR	0.031
Linear PFOS	0.015	NR	0.023	NR	0.023	0.018	0.023
Branched PFOS	0.013	NR	0.070	NR	0.070	0.018	0.070
PFOS	NA	0.095	NA	0.067	NA	NA	NA
PFNS	NR	NR	0.018	NR	0.018	NR	0.018
PFDS	NR	NR	0.001	NR	0.001	NR	0.001
4:2 FTSA	NR	NR	0.024	NR	0.024	NR	0.024
6:2 FTSA	0.093	NR	0.083	0.012	0.083	0.048	0.083
8:2 FTSA	0.023	NR	0.031	0.011	0.031	0.016	0.031
10:2 FTSA	NR	NR	0.001	NR	0.001	NR	0.001
6:2 FTAB	NR	NR	0.001	NR	0.001	NR	0.001
FBSA	0.021	NR	0.011	NR	0.011	0.023	0.011
FHxSA	0.021	NR	0.017	NR	0.017	0.046	0.017
FOSA	0.005	NR	0.012	0.002	0.012	0.012	0.012
FDSA	NR	NR	0.004	NR	0.004	NR	0.004
PFHxSAm	0.004	NR	0.004	NR	0.004	NR	0.004
PFHxSAmS	0.010	NR	0.010	NR	0.010	NR	0.010

¹Targeted PFAS data originally reported in Weber et al. (2017)

NR = not reported

NA = linear and branched isomers quantified separately

The total oxidizable precursor (TOP) assay. The total oxidizable precursor (TOP) assay was performed on groundwater samples following the procedure in our prior work (Weber et al. 2017; Ruyle et al 2021a; Ruyle et al 2021b). The procedure is slightly modified from the original method presented in Houtz and Sedlak (2012) in that it doubles the concentration of the potassium persulfate oxidant to enable complete oxidation of precursors. We combined equal parts by volume groundwater (2.5 mL) and 120 mM potassium persulfate (ACS-grade $K_2S_2O_8$, Honeywell, Charlotte, North Carolina, United States) and 250 mM sodium hydroxide (ACS-grade NaOH, Macron Fine Chemicals, Radnor, Pennsylvania, United States) in 15 mL PP centrifuge tubes (Corning, Corning, New York, United States) and heated for twelve hours at 85°C in a water bath. Samples were cooled to room temperature, neutralized with hydrochloric acid (ACS-Plus grade 37% HCl, Fisher Scientific, Hampton, New Hampshire, United States) to pH 6-8, and spiked with internal standard. Samples were loaded onto a weak anion exchange solid phase extraction cartridge (Waters Corporation Oasis® WAX, 6 mL, 150 mg sorbent, Milford, Massachusetts, United States) with a flowrate of one drop per second. After sample loading, cartridges were rinsed with 4 mL Milli-Q water and dried under 10 psi vacuum. PFAS were eluted into 15 mL PP centrifuge tubes with 4 mL LCMS grade methanol that was first used to rinse the sample bottles, followed by a rinse of 4 mL 0.1% ammonium hydroxide (28-30% ACS Grade, BDH Chemicals, Radnor, Pennsylvania, United States) in LCMS grade methanol. Extracts were blown to dryness using a N-EVAP (Organomation, Berlin, Massachusetts, United States) nitrogen evaporator, reconstituted in 750 mL LCMS grade methanol, vortexed, and heated at 40°C for 30 minutes. Samples were vortexed again and transferred to microcentrifuge tubes and mixed with 750 uL Milli-Q water before LC-MS/MS analysis using the same method as unoxidized samples. Concentrations of C3-C8 PFCA before and after the TOP assay are reported in **Table 11**.

The efficacy of precursor oxidation was evaluated by spiking 100,000 ng L⁻¹ of 4:2 FTSA, 6:2 FTSA, 8:2 FTSA, 10:2 FTSA, FBSA, FHxSA, FOSA, FDSA, 6:2 FTAB, N-MeFOSAA, and N-EtFOSAA in Milli-Q water before performing the TOP assay. Concentrations of each precursor declined by $\geq 99\%$ after the TOP assay indicating near complete oxidation. Additionally, concentrations of targeted precursors were <MDL in all oxidized samples except for 6:2 FTSA. Concentrations of 6:2 FTSA in oxidized samples were $\leq 10\%$ of its original concentrations in unoxidized samples.

We determined the concentrations of oxidizable precursors grouped by perfluorinated chain length and manufacturing origin (electrochemical fluorination [ECF] versus fluorotelomerization [FT]) using the Bayesian inference method developed in previous work (Ruyle et al. 2021a, Ruyle et al. 2021b). The method infers the original concentration distribution of precursors from changes in C3-C8 PFCA before and after the TOP assay (**Table 11**) from the yields of representative precursors reported in the literature and measured in our lab (Houtz and Sedlak 2012; Janda et al. 2019; Martin et al. 2019). Inclusion of measurements of PFCA < C3 has been previously shown to be unnecessary to infer precursors originating from AFFF because the method accounts for oxidization to PFCA < C3 and fluoride (Ruyle et al. 2021a). The original concentration distributions (**Error! Reference source not found.**) were estimated using Markov chain Monte Carlo (MCMC) analysis implemented in *emcee* 3.1.1 in Python 3.9.7. Samples from these distributions are generated using the differential evolution Snooker

algorithm with four walkers. We used a burn in window of 10,000 and sample until an effective sampling size of 2500 (MCMC error = $2500^{-0.5}$) is achieved.

The solution minimizes the least-squares of the log difference between the model and measurements of the TOP assay. We use a prior developed for AFFF-impacted systems for ECF-based precursors that was previously used to estimate precursors in the downstream watershed (Ruyle et al. 2021b). We implement a non-informative Jeffreys prior with variance equal to 5 for all FT precursors. We report the marginal posterior mean values of each precursor (**Table 12**) and use those values in all modeling and statistical analyses. A full mathematical description can be found in our previous works (Ruyle et al. 2021a,b) and the source code is available for download and use at <https://github.com/SunderlandLab/oxidizable-pfas-precursor-inference>.

Table 11. PFCA concentrations before and after TOP assay [nM].

PFCA	09/18/07	07/09/14	07/06/15	11/09/15	06/30/20	07/20/21
<i>Pre-oxidation</i>						
PFBA	5.03	1.01	2.32	1.41	3.35	0.69
PFPeA	13.24	2.24	5.18	3.32	8.55	1.27
PFHxA	23.99	3.19	9.48	6.71	16.06	1.75
PFHpA	7.59	1.26	5.36	3.35	5.04	0.42
PFOA	35.51	4.87	17.09	17.69	56.91	1.66
PFNA	0.08	0.00	0.00	0.07	0.05	0.00
<i>Post-oxidation</i>						
PFBA	25.04	15.93	15.45	19.73	29.44	4.09
PFPeA	27.74	12.87	12.15	18.74	30.46	4.86
PFHxA	69.75	33.49	25.80	36.24	76.39	16.76
PFHpA	9.30	2.32	5.24	4.18	6.52	0.79
PFOA	42.98	7.00	15.90	19.74	59.97	2.16
PFNA	0.15	0.11	0.07	0.10	0.12	0.05

*Values less than MDL are replaced by zero.

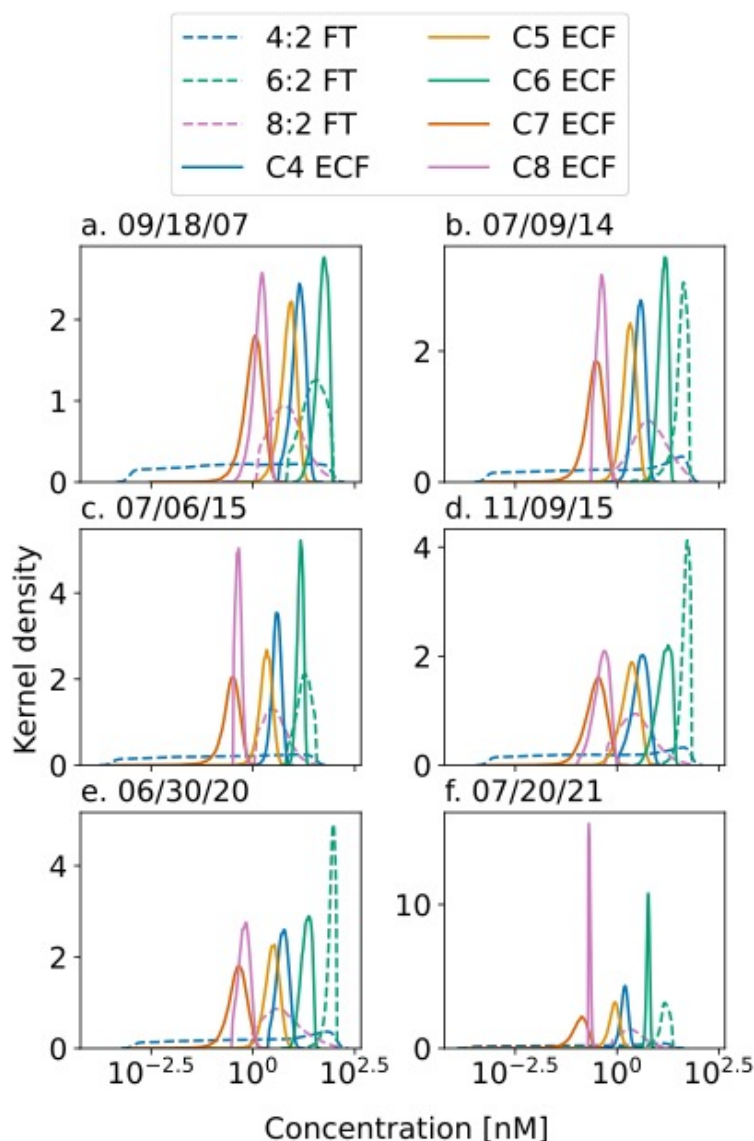


Figure 10. *Inferred concentrations of oxidizable precursors in groundwater.*

Panels show the marginal posterior probability density functions estimated by the nonparametric kernel density. A high kernel density indicates greater probability of the estimate. Precursors are grouped by perfluorinated chain length and manufacturing source. Electrochemical fluorination (ECF) precursors range from C4 to C8 while fluorotelomer (FT) precursors have n perfluorinated carbons followed by two aliphatic hydrocarbons ($n:2$, $n = 4, 6, 8$). Results on the different sampling dates between 2007 and 2021 are shown in panels a-f.

Table 12. Oxidizable precursor concentrations [nM].

	09/18/ 2007	07/09/ 2014	07/06/ 2015	11/09/ 2015	06/30/ 2020	07/20/ 2021
<i>Individual precursors</i>						
4:2 FT	0.46	0.60	0.22	0.47	1.29	0.21
6:2 FT	30.97	38.84	17.92	48.98	87.08	14.68
8:2 FT	6.39	6.23	3.36	2.84	4.87	2.24
C4 ECF	13.33	3.55	3.82	3.79	5.39	1.52
C5 ECF	7.66	2.02	2.10	2.13	2.99	0.85
C6 ECF	51.72	13.77	14.59	14.63	20.76	5.79
C7 ECF	1.05	0.28	0.30	0.31	0.42	0.12
C8 ECF	1.54	0.41	0.44	0.43	0.62	0.21
<i>Total precursors</i>						
Mean	126	73	46	80	135	28
Fluorine-normalized mean	1648	981	616	1051	1793	372
Fluorine-normalized standard deviation	343	177	117	164	293	59
ECF fraction [%]	89	78	84	78	76	79

Note: Values represent the mean of the marginal posterior distribution unless otherwise noted.

Extractable organofluorine (EOF). Groundwater (50 mL) was analyzed for extractable organofluorine (EOF) following the procedure established in prior work (Ruyle et al. 2021b). Samples were extracted similarly to the TOP assay with two differences: 1) internal standard was not added prior to extraction; and 2) inorganic fluoride was removed prior to drying the cartridge under vacuum by rinsing the cartridge with 10 mL 0.01% (v/v) ammonium hydroxide in Milli-Q water. We observed a >98% removal of a 2 mg L⁻¹ fluoride (NaF, 99+%, for analysis, ACROS Organics, Morris Plains, New Jersey, United States) spike in triplicate groundwater samples from 2021. Reconstituted samples (1 mL) were split evenly between analysis for EOF and PFAS on the LCMS. The LCMS split samples was spiked with internal standard and analyzed in the same manner as other samples and are reported in Table 13. The average relative percent difference of PFAS analysis for targeted analysis (no offline extraction) and EOF (internal standard added after offline extraction) was less than 25% for all PFAA except for PFOA (47%).

Table 13. Extractable organofluorine (EOF) concentrations [nM F].

	09/18/07	07/09/14	07/06/15	11/09/15	06/30/20	07/20/21
<i>Total</i>						
EOF	4029	1688	1472	2590	4611	654
Quantified PFAA	2774	637	998	1429	2964	214
Oxidizable precursors	1648	981	616	1051	1793	372
<i>Individual PFAS analytes</i>						
PFBA	27.1	6.4	11.8	7.9	19.3	<MDL
PFPeA	94.0	18.0	32.0	23.3	61.3	9.8
PFHxA	302.0	47.5	106.5	87.7	214.4	22.4
PFHpA	109.4	21.7	66.7	47.9	74.7	6.6
PFOA	851.3	139.6	341.1	427.5	1319.8	45.7
PFNA	2.3	1.5	<MDL	1.8	<MDL	0.7
PFDA	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
PFUnDA	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
PFDoDA	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
PFTTrDA	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
PFTeDA	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
PFBS	16.1	4.3	7.2	4.9	13.5	1.8
PFPeS	57.0	11.6	26.3	36.8	94.3	2.8
Linear PFHxS	555.9	168.5	235.2	508.3	846.8	43.8
Branched PFHxS	95.5	31.3	50.7	85.4	88.1	5.5
PFHpS	15.7	6.7	12.4	11.9	10.1	2.2
Linear PFOS	431.6	121.4	70.4	120.5	142.8	50.7
Branched PFOS	214.3	57.4	37.7	65.2	78.5	22.0
PFNS	1.6	1.0	<MDL	<MDL	<MDL	<MDL
PFDS	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
4:2 FTSA	<MDL	<MDL	<MDL	<MDL	0.9	<MDL
6:2 FTSA	69.0	25.7	56.9	65.7	204.1	6.9
8:2 FTSA	15.2	12.9	10.8	8.0	14.5	8.4
10:2 FTSA	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
6:2 FTAB	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
FBSA	15.1	2.3	6.5	3.6	9.2	0.8
FHxSA	77.7	18.8	14.0	13.9	21.8	8.9
FOSA	1.1	3.8	1.6	1.1	2.4	2.6
FDSA	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
PFHxSAm	NR	NR	NR	NR	NR	NR
PFHxSAmS	NR	NR	NR	NR	NR	NR

NR = not reported; <MDL = less than method detection limit

All samples for EOF determination were combusted at 1050°C with a combustion unit from Analytik Jena (Jena, Germany). Adsorption and detection were performed using a 920 Absorber Module and 930 Compact IC Flex ion chromatograph from Metrohm (Herisau, Switzerland). Two boat blanks were run between each set of duplicate injections of 100 mL methanol extracts and samples were blank corrected using the peak areas of the boat blanks run before and after each set of injections. Concentrations were determined from the average peak areas of the duplicate injections using a nine-point calibration curve ($R^2 = 0.999$) from 5 to 526 mM F. The relative standard deviation between duplicate peak areas was < 6% for all samples.

The limit of detection was 7 mM F and was calculated as the average plus three times the standard deviation of duplicate injections of an extraction blank of Milli-Q water that was processed simultaneously with the rest of the samples. Concentrations above the LOD were adjusted for the dilution factor (50x) and corrected by subtracting the extraction blank (**Table 13**). The concentrations of EOF in the samples were 9-288 times larger than in the extraction blank. The MDL was 0.05 mM F and was calculated as three times the standard deviation of the extraction blank. The relative percent difference for a set of field duplicates was 4% while the relative percent difference for a set of extraction duplicates was 1%. The method recovery (85%) was determined from a 26 mM F spike as PFOA (95% purity, Sigma-Aldrich, St. Louis, Missouri, United States) spike in groundwater.

Suspect screening. Suspect screening for 252 PFAS was performed using high-resolution mass spectrometry on raw groundwater samples. Chromatography was performed using an ExionLC (SCIEX, Framingham, Massachusetts, United States) system. Analytes were separated using a Phenomenex Gemini C18 column (110Å, 100 x 3 mm, 3 mm particle size; Torrance, California, United States). A delay column was used to separate PFAS compounds originating from the liquid chromatography. The mobile phases were water (A) and methanol (B), both with 10 mM ammonium acetate. The column oven was 40°C and the injection volume was 40 mL. Initial conditions were 20% B (0.5 min), increased to 55% B over 0.5 min, then increased to 99% B over 6 min and held for 1 min before returning to the initial condition. Analysis was performed on a SCIEX X500 series quadrupole time of flight (QTOF) mass spectrometer with a Turbo V ion source in ESI negative and positive mode. Data were collected using SWATH acquisition mode with a TOF MS experiment followed by 13 TOF MS/MS experiment using Q1 windows spanning 100 to 1000 Da. The TOF MS and each MS/MS experiment used an accumulation time of 50 ms, for a total cycle time of 0.8 s. SWATH experiments were conducted using a collision energy (CE) of 30/-30 V and a collision energy spread (CES) of ± 30 V. A broad CES was chosen to ensure comprehensive MS/MS spectra encompassing both high and low molecular weight fragments. SWATH acquisition windows were carefully chosen to avoid overlap with co-eluting analytes. The TOF MS scan ranged from 100 to 1000 Da using a DP = 50/-50V and CE = 5/-5V. The source and gas conditions were GS1 = 60, GS2 = 60, CUR = 35, CAD = 10, TEM = 500°C, ISV = 5500/-4500 V.

Data acquisition and data processing were performed using Analytics within the SCIEX OS software. Analytics links the precursor extracted ion chromatogram (XIC) with the appropriate MS/MS fragmentation pattern. Library matching was automatically performed using the SCIEX Fluorochemical HR-MS/MS library 2.0 which contains 252 PFAS. The MS/MS

spectra for positively matched compounds are shown in the accompanying supplementary file. We report the peak areas, mass errors (in ppm), and library fit score in **Table 14**, **Table 15**, **Table 16**.

Table 14. Peak areas of PFAS identified by suspect screening in ESI negative mode.

PFAS	09/18/07	07/09/14	07/06/15	11/09/15	06/30/20	07/20/21
PFPrS	8.26E+04	n.d. ¹	2.95E+04	1.75E+04	2.85E+04	n.d. ¹
PFBS	5.21E+05	1.28E+05	2.38E+05	1.63E+05	2.47E+05	5.10E+04
PFPeS	1.19E+06	2.88E+05	7.32E+05	6.96E+05	1.00E+06	6.71E+04
PFHxS	1.65E+07	6.95E+06	8.54E+06	1.38E+07	1.29E+07	1.32E+06
PFHpS	3.57E+05	1.96E+05	2.59E+05	2.38E+05	1.83E+05	4.58E+04
PFOS	2.03E+07	7.65E+06	2.97E+06	5.74E+06	8.23E+06	1.78E+06
PFPrA	4.58E+04	8.94E+03	1.90E+04	1.36E+04	2.09E+04	6.52E+03
PFBA	2.09E+05	4.22E+04	8.32E+04	5.53E+04	1.27E+05	2.17E+04
PFPeA	8.26E+05	1.56E+05	2.92E+05	2.14E+05	3.35E+05	7.03E+04
PFHxA	2.15E+06	3.55E+05	8.18E+05	7.05E+05	9.42E+05	1.47E+05
PFHpA	9.46E+05	2.26E+05	6.28E+05	4.47E+05	4.70E+05	5.10E+04
PFOA	8.17E+06	1.63E+06	3.05E+06	3.70E+06	7.90E+06	3.52E+05
PFNA	1.06E+04	1.32E+04	6.14E+03	5.65E+03	5.64E+03	3.44E+03
6:2 FTSA	3.84E+05	1.86E+05	2.85E+05	3.89E+05	8.42E+05	3.51E+04
FESA	1.92E+04	1.16E+04	2.95E+04	1.67E+04	3.72E+04	6.81E+03
FPrSA	2.23E+05	5.92E+04	1.35E+05	8.66E+04	2.01E+05	3.71E+04
FPeSA	4.33E+05	1.03E+05	9.17E+04	1.96E+05	2.67E+05	2.53E+04
FBSA	7.01E+05	9.08E+04	2.20E+05	1.91E+05	2.81E+05	2.91E+04
FHxSA	3.48E+06	8.64E+05	4.24E+05	6.34E+05	9.03E+05	3.45E+05
FOSA	2.89E+03	1.69E+05	3.57E+04	2.38E+04	4.89E+04	4.11E+04
N-MeFBSAA	8.62E+04	1.48E+04	3.43E+04	4.11E+04	8.11E+04	2.91E+03
N-SP-FBSA	1.88E+04	6.11E+03	2.38E+04	2.19E+04	2.47E+04	7.61E+02
N-SP-FHxSA	1.59E+05	9.67E+03	6.01E+03	1.60E+04	9.61E+03	3.45E+03
N-SPAmP-FBSA	2.20E+04	6.22E+04	3.06E+04	4.80E+04	3.20E+04	8.28E+03
N-SPAmP-FPeSA	5.02E+03	4.71E+03	4.00E+03	6.42E+03	1.19E+04	6.70E+03
N-SPAmP-FHxSA	2.35E+04	2.81E+04	1.76E+04	1.15E+04	3.26E+04	1.54E+04
N-SPAmP-FPeSAPS	3.19E+03	2.80E+04	1.31E+04	3.12E+04	1.22E+04	1.53E+03
N-SPAmP-FHxSAPS	3.38E+04	4.84E+04	1.69E+04	1.61E+04	3.01E+04	3.00E+04

¹n.d. = not detected

Table 15. Mass error (ppm) of PFAS identified by suspect screening.

PFAS	09/18/07	07/09/14	07/06/15	11/09/15	06/30/20	07/20/21
PFPrS	-3	n.d. ¹	0.5	1.4	-0.1	n.d. ¹
PFBS	-3.3	-0.3	0.0	-0.1	-0.7	-1.0
PFPeS	-2.2	-0.2	-0.4	-0.2	0.9	-0.8
PFHxS	-3.9	-0.5	0.8	0.0	-1.1	-1
PFHpS	-2.2	-0.7	-0.7	-0.1	0.4	-0.6
PFOS	-3.1	-1.1	0.4	0	-0.8	-0.6
PFPrA	-3.5	0.9	-0.5	-0.7	0.1	0.5
PFBA	-5.0	-1.6	-0.1	0.5	0.6	-0.4
PFPeA	-3.7	-0.2	-0.1	0.6	-0.5	0.5
PFHxA	-3.6	-0.8	-0.7	0	0.4	-0.9
PFHpA	-4.2	0.2	-0.2	0.9	-0.1	-0.9
PFOA	-1.1	-1.0	-0.5	-0.2	0.4	-0.6
PFNA	-1.0	0.1	-3.4	2.1	-1.6	-3.3
6:2 FTSA	-0.6	-0.6	-0.8	-0.2	0.9	1.0
FESA	-3.2	1.3	-0.5	0.7	0.3	-2.7
FPrSA	-3.6	0.6	0.0	0.9	0.0	-0.6
FPeSA	-4.9	-0.9	-0.9	0.4	-0.5	-0.6
FBSA	-3.6	0.2	-0.6	1.3	0.5	-0.1
FHxSA	-3.9	-0.5	-1.0	-0.6	0.1	-1.0
FOSA	2.5	-0.9	1.0	0.8	1.0	0.8
N-MeFBSAA	-2.0	-3.2	0.3	-1.0	0.8	0.7
N-SP-FBSA	0.6	-0.6	-0.8	-0.1	-0.6	-4.4
N-SP-FHxSA	-1.5	-0.4	-7.6	-2.0	-1.8	-5.3
N-SPAmP-FBSA	-0.9	0.7	-0.9	0.9	0.7	-0.8
N-SPAmP-FPeSA	-0.9	-0.6	0.6	0.6	1.6	-2.6
N-SPAmP-FHxSA	1.5	1.0	0.3	-1.8	0.2	-0.2
N-SPAmP-FPeSAPS	-9.8	0.6	0.0	-0.5	1.0	3.1
N-SPAmP-FHxSAPS	-3.7	-1.7	1.4	-1.6	8.5	-2.0

¹n.d. = not detected; The mass error of the X500 QTOF is rated as 5ppm.

Table 16. Library hit score (fit) of PFAS identified by suspect screening.

PFAS	09/18/07	07/09/14	07/06/15	11/09/15	06/30/20	07/20/21
PFPrS	100	n.d. ¹	98	99	100	n.d. ¹
PFBS	100	100	100	98	98	99
PFPeS	100	100	100	100	100	100
PFHxS	100	100	100	100	100	100
PFHpS	99	99	98	98	99	99
PFOS	100	100	100	100	100	100
PFPrA	99	99	99	99	99	99
PFBA	100	100	100	100	100	99
PFPeA	100	100	100	100	100	100
PFHxA	100	100	100	100	100	100
PFHpA	100	100	100	100	100	100
PFOA	100	100	100	100	100	100
PFNA	100	99	100	96	95	0
6:2 FTSA	100	100	100	100	100	100
FESA	64	73	64	0	62	0
FPrSA	100	100	100	100	100	99
FPeSA	100	100	100	100	100	100
FBSA	100	100	100	100	100	100
FHxSA	100	100	100	100	100	100
FOSA	100	100	100	100	100	100
N-MeFBSAA	67	60	0	91	65	0
N-SP-FBSA	100	98	99	100	98	86
N-SP-FHxSA	100	100	88	93	100	99
N-SPAmP-FBSA	97	96	99	96	99	92
N-SPAmP-FPeSA	96	97	92	96	98	96
N-SPAmP-FHxSA	95	94	93	80	94	99
N-SPAmP-FPeSAPS	0	100	99	100	99	99
N-SPAmP-FHxSAPS	97	62	97	98	99	99

¹n.d. = not detected; Values >70% indicate a good fit.

Model description. We developed a simple 4-box geochemical model to represent the temporal evolution of precursors and changes in reservoirs of the terminal PFAS in soils and groundwater at the former fire-training area over time. The model includes individual compartments for precursors and terminal compounds in soil and groundwater (**Figure 11, Equations 10-13**). It is forced by inputs from AFFF-use (**Equation 14**), constrained by known dates of use and measurements of PFAS in soil cores at the fire-training area, and in groundwater over time. We use uniform priors to characterize plausible uncertainty of AFFF use (PFAS loading), precursor biodegradation rates, and retardation due to partitioning to solids using data obtained from the literature and column experiments as part of this study (**Table 17**). Advection rates through soil and groundwater were based on hydrological information from USGS (**Table 17**). We then optimized the simulation (PFAS loading, biodegradation rate, and retardation) to reproduce

measured values with minimum uncertainty (differences between modeled and observed soil and groundwater concentrations) using Bayesian inference. Specifically, we sample from the data-optimized distribution of parameters using MCMC with the same sampler and hyperparameters that were used to infer oxidizable precursor concentrations from the TOP assay.

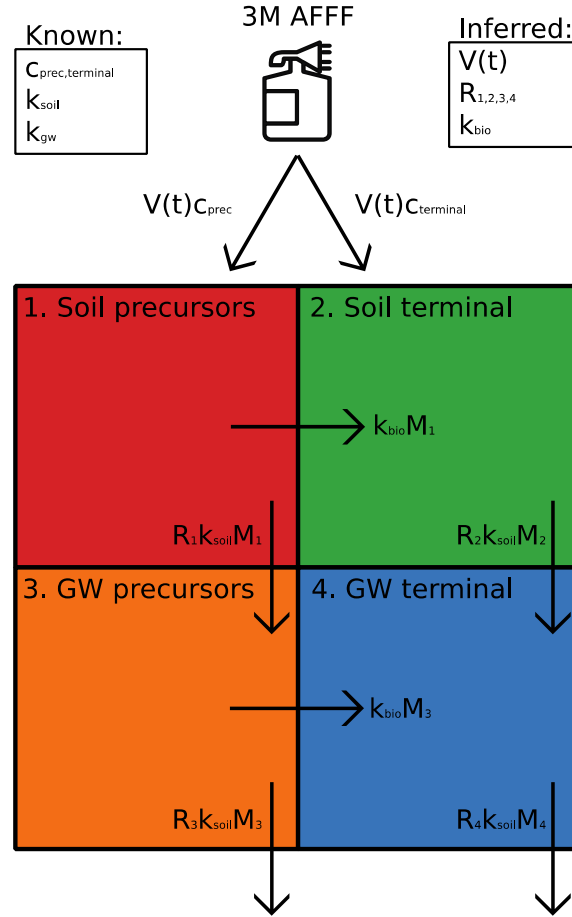


Figure 11. Conceptual four-box model for the former fire training area.

Known parameter values and prior bounds specifying the plausible uncertainty for inferred parameters are derived from the literature and experimental work as a part of this study (Equations 10-14).

Model algorithms.

$$\frac{dM_1}{dt} = V(t)C_{prec} - k_{bio}M_1 - R_1k_{soil}M_1 \quad (\text{Equation 10})$$

$$\frac{dM_2}{dt} = V(t)C_{terminal} + k_{bio}M_1 - R_2k_{soil}M_2 \quad (\text{Equation 11})$$

$$\frac{dM_3}{dt} = R_1k_{soil}M_1 - k_{bio}M_3 - R_3k_{GW}M_3 \quad (\text{Equation 12})$$

$$\frac{dM_4}{dt} = R_2 k_{\text{soil}} M_2 + k_{\text{bio}} M_3 - R_4 k_{\text{GW}} M_4 \quad (\text{Equation 13})$$

$$V(t) = \begin{cases} V_{\text{training}} & \text{if } 1970 \leq t \leq 1985 \\ V_{\text{fire}} & \text{if } t = 1997 \\ 0 & \text{otherwise} \end{cases} \quad (\text{Equation 14})$$

Where:

M_i = mass of PFAS in compartment i [mol]

$V(t)$ = the yearly volumetric application of AFFF [L yr⁻¹] (inferred)

c_{prec} = the concentration of precursor in 3M AFFF [M] (Table S7 in Ruyle et al. 2021a)

c_{terminal} = the concentration of terminal PFAA in 3M AFFF [M] (Table S7 in Ruyle et al. 2021a)

k_{bio} = precursor biodegradation rate [yr⁻¹] (inferred)

$k_{\text{soil}} = 10$ = rate of water advection through soil [yr⁻¹] (Massey et al., 2006)

$k_{\text{GW}} = 1$ = rate of water advection through groundwater [yr⁻¹] (Garabedian et al., 1991)

R_i = PFAS retardation factor in compartment i (inferred)

Table 17. Model* parameters for four-box model.

<i>Known parameters</i>				
k_{soil} [yr ⁻¹]	10			
k_{gw} [yr ⁻¹]	1			
	C7 PFCA	C4 PFSA	C6 PFSA	C8 PFSA
c_{terminal} [mM]	0.6	0.8	4.1	17.5
c_{prec} [mM]	NA	5.2	17.7	0.5
<i>Inferred parameters</i>				
V_{training} [L yr ⁻¹]	75.6 – 2726.4			
V_{fire} [L yr ⁻¹]	18.9 – 454.4			
	C7 PFCA	C4 PFSA	C6 PFSA	C8 PFSA
$R_{\text{soil,precursor}}$	NA	4.6 – 1100	10.9 – 1100	50 – 1100
$R_{\text{soil,terminal}}$	2 – 1100	1.1 – 1100	1.3 – 1100	11.9 – 1100
$R_{\text{GW,precursor}}$	NA	1 – 8.2	1 – 1.7	25.5 – 74
$R_{\text{GW,terminal}}$	1.4 – 2.6	1 – 1.2	12.3 – 32.9	6.2 – 17.7
k_{bio} [yr ⁻¹]	NA		1E-5 – 1	

*Model schematic and algorithms are shown in **Figure 11** and **Equations 10-14**.

Mass Reservoirs. Concentrations of terminal PFAS in groundwater (**Table 9**) were converted to mass reservoirs (**Table K**) by: (1) determining the 2018 reservoir size by multiplying the length of the fire training area [150 m] by the interpolated cross-sectional concentration [mol m⁻¹] from a series of four profiles (0-14 m below the water table) spanning the width of the fire training area sampled by the AFCEC in 2018 (Unpublished Technical Updates, Personal Communications, Rose Forbes, AFCEC); and (2) determining the groundwater reservoir size between 2007-2021 by multiplying the 2018 reservoir size by the ratio of groundwater concentrations reported here and the shallowest two points of the nearest profile. The groundwater we collected is 11 m upgradient from the nearest profile (~70 days groundwater

transport time) and samples the same depth as the two shallowest points. Interpolation was performed using *scipy* version 1.7.1. Concentrations of terminal PFAS in soil were converted to mass reservoir by multiplying the reported concentration by its representative volume (Unpublished Technical Updates, Personal Communications, Rose Forbes, AFCEC).

Table 18. Terminal PFAS reservoirs beneath the fire training area [mM].

Year	Source	Reservoir	C7 PFCA	C4 PFSA	C6 PFSA	C8 PFSA
2007	This study	GW	1521	90	2635	12324
2014	Weber et al.	GW	250	32	925	3585
2014	This study	GW	208	18	597	2935
2014	AFCEC	GW	467	58	926	10563
2015	Weber et al.	GW	732	52	1485	2769
2015	This study	GW	758	28	1961	3795
2015	AFCEC	GW	1016	40	1832	6671
2019	AFCEC	GW	2386	114	3085	8895
2020	This study	GW	2438	68	2750	4711
2020	AFCEC	GW	4253	92	1928	5559
2020	NIST	GW	2003	70	2306	4435
2021	This work	GW	71	9	170	936
2018	AFCEC	Soil	8368	21	4046	35000

This study: data reported in this work

Weber et al.: Weber et al. (2017)

AFCEC: Unpublished Technical Updates, Personal Communications, Rose Forbes, AFCEC.

NIST: data reported by the National Institute for Standards and Technology Hollings Marine Laboratory in personal communications with Dr. Alix Robil and Dr. Katherine T. Peter

Site specific sediment-water (K_d) and air-water interface (K_{ai}) partitioning. We calculated site specific K_d and K_{ai} using retardation factors inferred from the model according to Brusseau et al. (2018):

$$R_{GW} = 1 + K_d \rho_b / \theta_w \quad \text{Equation 15}$$

$$R_{soil} = 1 + K_d \rho_b / \theta_w + K_{ai} A_{ai} / \theta_w \quad \text{Equation 16}$$

Where:

R_{GW} = advective transport retardation coefficient in groundwater [unitless]

K_d = sediment-water partition coefficient [mL g^{-1}]

ρ_b = soil/sediment bulk density = $1.63 \text{ [g cm}^{-3}\text{]}$

θ_w = volumetric water content [unitless] = 0.39 in groundwater and 0.125 in soil¹

K_{ai} = air-water interface partition coefficient [cm^{-1}]

A_{ai} = specific air-water interfacial area [cm] = 929.712^2

¹In soil, we estimate the steady state θ_w to be 0.125 as the porosity (0.39) (Garabedian et al., 1991) minus the specific yield (0.265) (Moench et al., 2001).

²We estimate the steady state A_{ai} from the steady state θ_w following the empirical equation proposed in Brusseau et al. (2006) The equation hyperparameters (a , b , c , α) are not known for our field site. However, the mineralogy and sediment characteristics on Cape Cod are nearly identical to those used in Brusseau et al. (2006). Therefore, we use their reported hyperparameters to estimate A_{ai} .

Task 4 Methods: Passive samplers

Chemicals and reagents. Liquid chromatography-grade methanol (LC-MeOH), and water (LC-water) were purchased from Fisher Scientific (New Hampshire, USA) along with ammonium hydroxide (NH₄OH), ammonium acetate (C₂H₇NO₂), ACS-grade ethanol (EtOH) and ACS-grade methanol (MeOH). Analytical standards were used to create native compound standards from the Wellington PFAC-30PAR mix plus an additional four analytical compounds (Table S1). Mass labeled surrogate solutions were derived from Wellington Laboratories' MPFAC-30ES plus an additional three mass labeled compounds purchased from Wellington Laboratories (Canada). Standards with a range of purity (94-98%) were used to create spiking solutions for all laboratory experiments and consisted of twenty-four individual compounds purchased from Toronto Research Chemicals (Canada), SynQuest (Florida, USA), Apollo Scientific (United Kingdom), and Santa Cruz (Texas, USA) (S1). Solutions were created from solid standards and diluted in LC-MeOH before being stored in the refrigerator while awaiting use. Only 9 PFAA compounds (the CX-CX PFCA and the CX-CX PFSA) have published aqueous diffusivities, an important parameter for modeling sampler uptake. These 9 PFAAs were included in the twenty-four mix spiked into the experimental apparatus and are the focus of this study. Two specific PFAA will be highlighted, PFHxA and PFHxS, as they have become broadly used in place of PFOA and PFOS and are detected in many environmental matrices. Three additional PFAS compounds, two long chain (>C7) PFCAs and one fluorotelomer sulfonate were also included in the study.

Passive Sampler Assembly. The assembly of the novel tube passive samplers for PFAS was based on the method outlined by Fauvelle et al. (2017). Microporous polyethylene tubes purchased from the Pall Corporation (Filtroplast, 12 mm O.D., 8 mm I.D. 35% porosity, 2.5 μ m pore size, 0.6 g cm⁻¹ density) were cut into 7 cm long sections. These 7 cm long tube sections were then submerged in a 24-hour EtOH wash which was followed by a 24-hour LC-MeOH wash. Once fully dry, one end of the tube was closed using a snap-in polyethylene cap purchased from McMaster Carr, and 600 mg of Oasis hydrophilic lipophilic balance (HLB) sorbent was carefully measured out and poured inside the open end of the tube. Prior to deployment in the laboratory, assembled tube samplers were conditioned for at least 24 hours in LC-MeOH, followed by 24-hours in LC-water. The tube samplers were then either immediately deployed into the laboratory, field, or stored in LC-water until the experiment began.

Passive Sampler Extraction. Immediately following deployment, the tube passive sampler was placed in a 15 mL Falcon Tube and centrifuged at 4000 rpm for 2 minutes using an Eppendorf Centrifuge 5810 (Fisher Scientific, USA) to remove any water remaining in the tube walls or sorbent. This centrifugation step is repeated until no further water is collected in the bottom of the tube. At this point, the tube was either frozen for future extraction after a maximum of six months or extracted immediately using liquid solid extraction (LSE). Tube samplers were submerged in approximately 6 mL of LC-grade MeOH within a 15 mL Falcon tube and spiked with a mass labeled surrogate standard (MPFAC-30ES+3). After 24 hours, this methanol extract was collected in a pre-labeled, pre-cleaned Falcon tube. The tube sampler was centrifuged as previously described to remove any additional LC-MeOH, which was added to the collected extract. The extraction step was then repeated, resulting in roughly 12 mL of extract. The extract was concentrated to approximately 0.5 mL in total volume under a gentle stream of Nitrogen while heated to 35°C. An aliquot of this final concentrate was diluted with 4 mM Ammonium

Acetate in water and prepared for analysis via liquid chromatography tandem mass spectrometry (LC/MS/MS)

Instrumental Analysis. LC/MS/MS analysis was performed at the University of Rhode Island on a Shimadzu Prominence liquid chromatograph (UFLC) coupled to SCIEX TripleQuad 5500 MS/MS operating in negative mode for all laboratory and most field samples. Field samples collected in on Cape Cod were analyzed at Harvard University using an Agilent 6460 Triple Quadrupole Liquid Chromatography-tandem Mass Spectrometer following the method in previous work (Ruyle et al., 2021b). Additional details on the operating conditions, quality assurance, and interlaboratory comparison are provided in **Table 19**, **Table 20**, **Table 21**, **Table 22**.

Table 19. LC conditions For University of Rhode Island.

Mobile phase / time (min)	A (%) – 10 mM ammonium acetate in water	B (%) – 10 mM ammonium acetate in methanol
0.0	60	40
0.5	60	40
3.5	45	55
10.0	15	85
13.0	15	85
13.1	60	40
16.0	60	40

Table 20. Mass spectrometer (MS) instrumental parameters for University of Rhode Island.

Curtain Gas (CUR)	30
Collision Gas (CAD)	medium
IonSpray Voltage (IS)	-4500 V
Temperature (TEM)	400°C
Ion Source Gas 1 (GS1)	30
Ion Source Gas 2 (GS2)	30

Table 21. MS/MS Parameters of target PFAS for University of Rhode Island.

Analyte	Type	Internal Standard	MRM transitions	DP *(V)	CE** (V)
PFBA	Target	MPFBA	213 → 169; -	-20	-12; -
PFPeA	Target	M5PFPeA	263 → 219; -	-40	-10; -
PFHxA	Target	M5PFHxA	313 → 269; 119	-35	-13; -26
PFHpA	Target	M4PFHpA	363 → 319; 169	-55	-15; -24
PFOA	Target	M8PFOA	413 → 369; 169	-45	-17; -24
PFNA	Target	M9PFNA	463 → 419; 219	-40	-15; -24
PFDA	Target	M6PFDA	513 → 469; 219	-50	-18; -26
PFUnDA	Target	M7PFUnDA	563 → 519; 269	-45	-20; -26
PFDoDA	Target	M2PFDoA	613 → 569; 319	-45	-22; -26
PFTTrDA	Target	M2PFDoA	663 → 619; 169	-50	-19; -36
PFTeDA	Target	MPFTeDA	713 → 669; 169	-65	-22; -34
PFBS	Target	M3PFBS	299 → 80; 99	-45	-70; -40
PFPeS	Target	M3PFBS	349 → 80; 99	-50	-65; -50
PFHxS	Target	M3PFHxS	399 → 80; 99	-55	-60; -45
PFHpS	Target	M3PFHxS	449 → 80; 99	-50	-100; -85
PFOS	Target	M8PFOS	499 → 80; 99	-55	-120; -90
PFNS	Target	M8PFOS	549 → 80; 99	-50	-120; -100
PFDS	Target	M8PFOS	599 → 80; 99	-45	-120; -115
PFECHS	Target	M8PFOS	461 → 381; 99	-45	-40; -40
4:2 FTSA	Target	M2-4:2FTS	327 → 307; 81	-50	-28; -40
6:2 FTSA	Target	M2-6:2FTS	427 → 407; 80	-50	-32; -42
8:2 FTSA	Target	M2-8:2FTS	527 → 507; 71	-50	-40; -46
FBSA	Target	MFOSA	298 → 78; 64	-40	-50; -90
FOSA	Target	MFOSA	498 → 78; 64	-105	-84; -130
MeFOSA	Target	d-N-MeFOSA	512 → 169; 219	-95	-36; -34
EtFOSA	Target	d-N-EtFOSA	526 → 169; 219	-90	-36; -34
N-MeFOSAA	Target	d3-N-MeFOSAA	570 → 419; 483	-40	-36; -30
N-EtFOSAA	Target	d5-N-EtFOSAA	584 → 419; 526	-50	-36; -30

*DP – declasteration potential

**CE – collision energy

Table 22. Interlaboratory* comparison of PFAS in surface water from the Quashnet River.

ng/L	HU	URI	GEL
PFBA	3.5	4.8	4.0
PFPeA	8.3	10	12
PFHxA	12	13	16
PFHpA	5.2	5.6	8.1
PFOA	11	11	18
PFNA	6.0	7.0	9.6
PFDA	0.06	0.15	<0.69
PFBS	3.3	4.0	4.2
PFHxS	37	53	53
PFHpS	1.4	0.91	1.1
PFOS	46	76	73
6:2 FTS	5.6	3.4	7.9

*HU = Harvard University (HU); URI = University of Rhode Island; GEL = DoD ELAP-Accredited Gel Labs (GEL).

Experimental Tank Design. To create artificial environmental conditions for the calibration and modeling of the novel tube passive sampler an experimental tank design was adapted from Urik and Vrana (2019) and modified using PFAS-friendly materials. A custom high density polyethylene tank (100 cm L x 40 cm W x 30 cm H) was designed and purchased from PKG Equipment (Rochester, New York) to minimize sorption of PFAS to the tank walls. The tank was black and featured a removable cover, to prevent light from penetrating into the experiments to limit any transformation of PFAS compounds via photolysis or growth of bacteria and algae within the water. Three submersible aquarium pumps (KEDSUM, Zhejiang, China) were used to circulate the water around the baffle (70 cm L x 3 cm W x 30 cm H) within the tank, which was located slightly off center, to create a large experimental flow channel for the deployment of multiple tube passive samplers directly in front of the pumps (**Figure 12**).



Figure 12. Schematic of experimental tank.

Locations of the three submersed pumps in relation to the passive samplers and baffle used to create two channels for circulation.

Experimental Tank Deployments and Measurements. To measure laboratory uptake and sampling rates, triplicate tube passive samplers were deployed at four different flow rates (0, 10, 20, and 60 cm s⁻¹) and two different temperatures (15°C and 25°C). Each trial consisted of a 14-day deployment, where duplicate 50 mL water grab samples were taken at the beginning and end of each deployment to verify PFAS concentrations during deployments. Water samples were extracted and analyzed in accordance with methods detailed in literature and used to calculate sampling rates for corresponding individual passive samplers (Becanova et al., 2021, Morales-McDevitt et al., 2022).

PFAS Uptake by HDPE Membranes and HLB Sorbent. As part of the validation process of this passive sampler design and model, partitioning was measured for PFAS between the microporous polyethylene membrane and water (K_{mw}). K_{mw} values were calculated as the mass of a given PFAS compound per gram of polyethylene divided by the mass of a given PFAS per gram of water at two temperatures (15 and 25 °C) and high flow rates (20-60 cm s⁻¹) for month-long deployments. To accomplish this, the microporous membrane tubes were prepared as detailed above, but without the addition of any sorbent. Thus, any uptake measured in the extraction of the sampler can be attributed to sorption to the microporous polyethylene walls (for details, see the SI).

Field Deployments. Passive samplers were deployed in the field for 14-28 days at three locations, including AFFF-impacted groundwater (JBCC) and river (QR9) downstream of a former fire training area on Joint Base Cape Cod, MA and a river downstream of a historical textile mill in Westerly, RI (Pawcatuck). Groundwater deployments were conducted for 14 days in June 2021 in a previously characterized PFAS plume (Weber et al., 2017) where the average linear groundwater velocity is 5x10⁻⁴ cm s⁻¹ (Garabedian et al., 1991). River deployments occurred between October 2019 and September 2020 at QR09 and in May 2021 at Pawcatuck. All surface water measurements ranged from 50-70 cm s⁻¹ approximate flow speed based on estimations from USGS gage station 0111058837 for QR9 or from handheld measurements using a flow meter in the Pawcatuck River. Grab samples of surface and groundwater were collected during deployment and/or recovery of passive samplers and extracted in the manner described in previous literature by either the University of Rhode Island or Harvard University (Becanova et

al., 2021; Ruyle et al., 2021b; Morales-McDevitt et al., 2022). An inter-lab comparison of the water from QR9 was performed between the two labs and a DoD certified private lab (Table 22).

Uptake by Bio Fouled Tube Membranes. Field deployments might incur an additional resistance to uptake from biofouling that is not accounted for in most laboratory and model methods. Previously bio fouled tubes were deployed in the experimental tank alongside freshly prepared tubes at two similar temperatures and two distinct flow speeds (0 and 60 cm s⁻¹) to investigate potential interferences of biota and environmental detritus on uptake through the microporous membrane.

Validation of a Numerical Model. Aqueous diffusion coefficients for 9 PFAAs reported by (Schaefer et al. 2019) were used to calculate mass transfer coefficients (MTC) through the water boundary layer, microporous membrane layer, and sorbent layer (Schaefer et al, 2019). It is assumed that transport is entirely through the pore space of the microporous membrane and that the only diffusion within the sorbent layer is from aqueous diffusion within the water saturated interior. The basic concept of estimating mass transfer in this manner has been suggested in previous literature on integrative passive sampling (Vrana et al., 2005).

RESULTS AND DISCUSSION

Task 1 Results and Discussion: PFAA and precursor mobility in groundwater

Site-specific log K_d values for PFAS in Cape Cod sediment ranged from -1.7 to 0.6 (Figure 13). Preference for the solid phase generally increased with the number of perfluorinated carbons except for C3-C5 PFCA, which were conservatively transported (the C4 PFSA was also conservatively transported). Each additional perfluorinated carbon was associated with a 0.72 ± 0.05 -log increase in K_d averaged across all PFAS classes. For the same number of perfluorinated carbons, sorption also depended on the functional group and followed the order PFCA < PFSA < FTSA < FASA (Figure 13). Lab-derived K_d values agreed with field-derived K_d reported by Weber et al. (2017) within an order of magnitude (Table 23).

Table 23. Joint Base Cape Cod (JBCC) field-site sediment-water partition coefficients.

PFAS	Log (K_d) estimated from breakthrough method ¹ (mean $\pm$ st. dev)	Log (K_d) estimated from Hydrus1D (mean $\pm$ st. dev)	Log (K_d) reported in Weber et al. (2017) (mean $\pm$ st. dev)
PFBA	-1.67 $\pm$ 0.02	Not estimated ⁴	Not reported
PFPeA	-1.59 $\pm$ 0.06	Not estimated ⁴	-1.65 $\pm$ 0.77
PFHxA	-1.67 $\pm$ 0.05	Not estimated ⁴	-1.26 $\pm$ 0.17
PFHpA	-1.39 $\pm$ 0.11	-1.23 $\pm$ 0.03	-1.06 $\pm$ 0.08
PFOA	-0.66 $\pm$ 0.13	-0.61 $\pm$ 0.10	-1.11 $\pm$ 0.55
PFBS	-1.61 $\pm$ 0.10	Not estimated ⁴	Not reported
PFPeS	-1.55 $\pm$ 0.21	Not estimated ⁴	Not reported
Linear PFHxS	-1.20 $\pm$ 0.26	-1.02 $\pm$ 0.02	-1.50 $\pm$ 0.16 ⁵
Branched PFHxS	-1.30 $\pm$ 0.21	-1.14 $\pm$ 0.03	
PFHpS	-0.30 $\pm$ 0.14	-0.28 $\pm$ 0.11	Not reported
Linear PFOS	0.41 $\pm$ 0.15	0.43 $\pm$ 0.13	-0.36 $\pm$ 0.17 ⁵
Branched PFOS	0.19 $\pm$ 0.09	0.18 $\pm$ 0.11	
6:2 FTSA	-0.89 $\pm$ 0.34	-0.75 $\pm$ 0.14	-1.10 $\pm$ 0.93
8:2 FTSA	0.62 $\pm$ 0.07	0.73 $\pm$ 0.06	0.02 $\pm$ 0.41
FBSA	-0.14 $\pm$ 0.20		Not reported
FHxSA	0.33 ²		Not reported
FOSA	Not estimated ³	Not estimated ³	1.04

¹From equation 2.24 from citation ⁷ [$K_d = (R_f - 1) / (\phi / \rho_b)$] where R_f is the retardation factor, ϕ is the porosity, and ρ_b is the bulk density

²Breakthrough of FHxSA was only observed in one of three replicate columns

³Breakthrough of FOSA was not observed in any of the replicate columns

⁴PFAS with breakthrough pore volume times ≤ 1.1 were not modeled using Hydrus1D

⁵Sum of linear and branched isomers

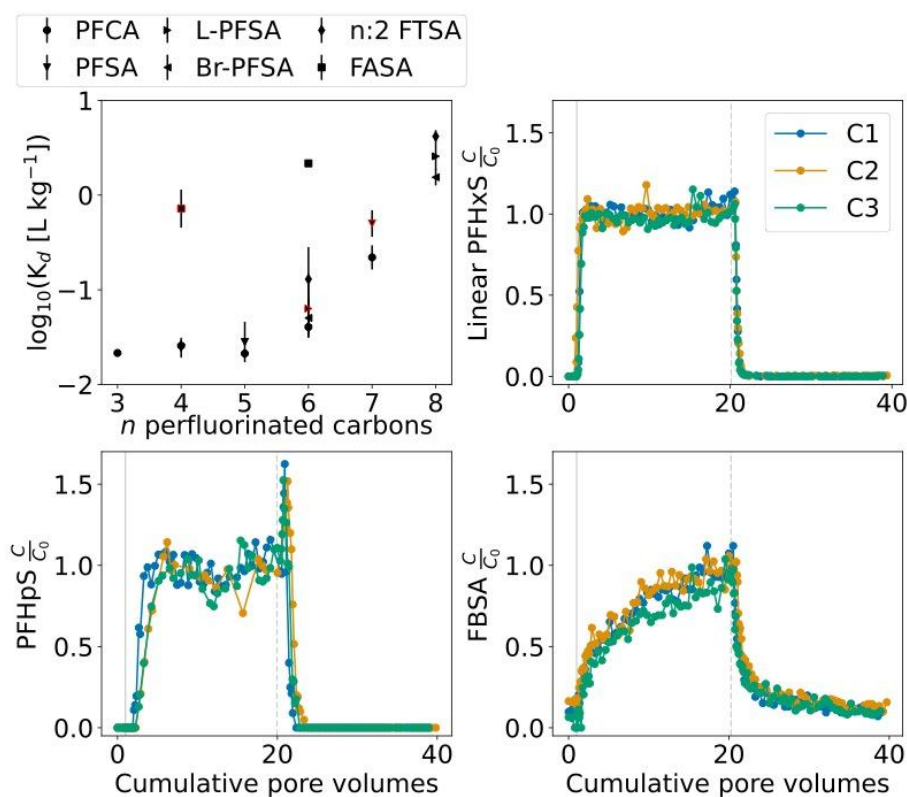


Figure 13. Trends in sediment-water partitioning (K_d).

Results are based on the number of perfluorinated carbons and functional group along with breakthrough curves from representative PFAS.

A spike in aqueous-phase concentration was observed for the PFAS with $\log K_d$ values > -1 , except for FASA, as the columns were eluted with PFAS-free reference groundwater (**Figure 13, Figure 14**). No spike in aqueous-phase concentration was observed for the C3-C6 PFCA, C4 – C6 PFSA, and FASA. The maximum observed spike in concentration ranged from 120 – 200% of the input level. Spikes in concentration above the input level are the result of oversaturation of the solid phase and demonstrate that sediment-water partitioning was sensitive to changes in water chemistry between the two groundwaters (

Table 1). Values for K_d were greater in groundwater from the fire training area by 0.16 ± 0.04 log units compared to groundwater from the reference site.

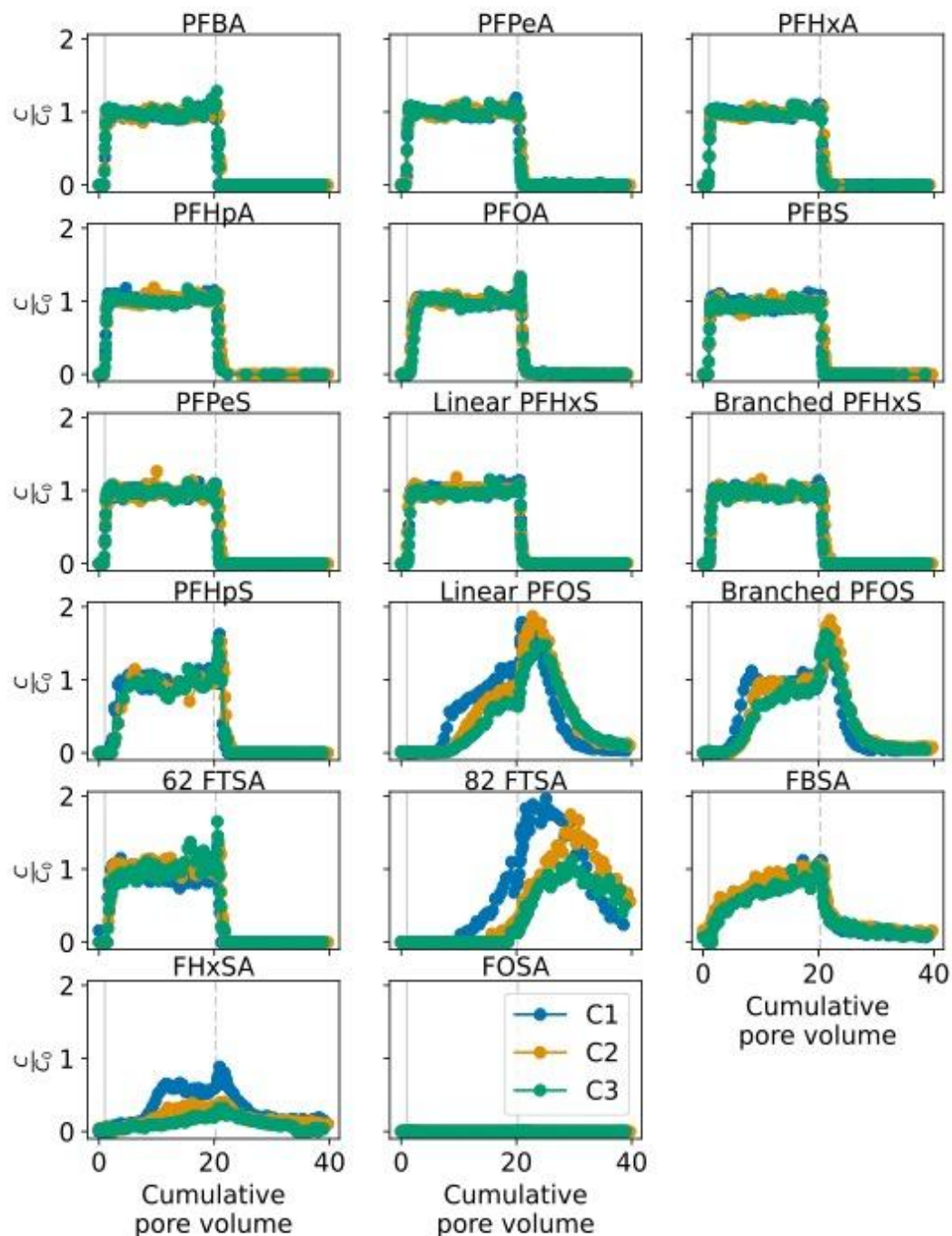


Figure 14. PFAS breakthrough curves normalized to influent concentration (

Table 1).

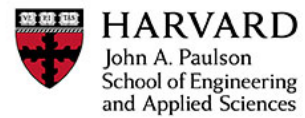
The solid gray line indicates one pore volume of the loading phase (and the first pore volume of the experiment). The dashed gray line indicates one pore volume of the eluting.

Previous work has shown predominant water chemistry factors that affect PFAS sorption include pH, dissolved organic carbon (DOC), salinity, and divalent cation concentrations (Higgins and Luthy, 2006; Du et al., 2014). The decline in sorption during the elution phase for PFAS with $K_d > 1$ (**Table 24**) was unlikely due pH and DOC because opposite trends from the literature were observed (Du et al., 2018). Changes in sorption were also unlikely due to conductivity because the salting out effect is not significant at the low conductivities ($< 125 \mu\text{S cm}^{-1}$) of the two groundwaters (You et al., 2010; Munoz et al., 2017). Therefore, the effect of divalent cations, which neutralizes, and bridges negatively charged surface sites and anionic PFAS, is the most plausible mechanism driving sorption in the experiment. A log-unit increase in the sum of calcium (Ca^{2+}) and magnesium (Mg^{2+}) associated with a 0.21 ± 0.06 log-unit increase in PFAS sorption. Observed changes in sorption of PFCA, PFSA, and FTSA were similar across functional groups, consistent with larger changes in Ca^{2+} (which can bridge all compounds due to its lower electronegativity) versus Mg^{2+} (which preferentially bridge carboxylates due to its higher electronegativity) between the two groundwaters (Wang et al., 2011; Kennedy et al., 2004). The role of divalent cation bridging which affects only deprotonated PFAS may also explain why no spike in concentration was observed for the FASA, which are expected to be only 3 – 27% anionic in the range of pH of the groundwaters (pK_a estimated by ARChem: FBSA = 6.59, FHxSA = 6.57) (ArChem, 2010).

Divalent cation bridging has significant field implications for PFCA retardation because large increases in divalent iron and manganese (which are both more electronegative than calcium) are observed in reducing regions of the plume (Weber et al., 2017). Divalent cation complexation of carboxylates is stronger than for sulfonates due to their lesser degree of hydration (Groenendijk et al., 2021). We hypothesize that reducing conditions observed in infiltrating groundwater from a downgradient lake could explain greater sorption of PFCA compared to PFSA in Tokranov et al. (2021). Future work should systematically explore the effect of sorbent electronegative (Ca^{2+} , Mg^{2+} , Fe^{2+} , and Mn^{2+}) and sorbate (PFCA vs. PFSA vs. FASA) on divalent cation bridging.

Table 24. Sediment-water partition coefficients of column experiment loading and eluting phase.

PFAS	loading log (K_d) estimated from Hydrus1D (mean $\pm$ st. dev)	eluting log (K_d) estimated from Hydrus1D (mean $\pm$ st. dev)
PFOA	-0.61 ± 0.10	-0.76 ± 0.05
PFHpS	-0.28 ± 0.11	-0.51 ± 0.04
Linear PFOS	0.43 ± 0.13	0.26 ± 0.12
Branched PFOS	0.18 ± 0.11	0.03 ± 0.06
6:2 FTSA	-0.75 ± 0.14	-0.85 ± 0.06
8:2 FTSA	0.73 ± 0.06	0.58 ± 0.03



Task 2 Results and Discussion: Precursor biotransformation

Rapid biological association of sulfonamido precursors. Large and rapid losses of the major precursors in the 2001 3M AFFF (**Figure 15**: PFHxSAm, PFHxSAmS, and FHxSA) from the aqueous phase were observed immediately after addition ($t=0$) in both the bioactive and control microcosms, indicating rapid sorption occurred in all experiments (**Figure 16**; **Figure 17**). Greatest losses were observed in the bioactive slurries, followed by the abiotic slurries, and DI controls with no sediment.

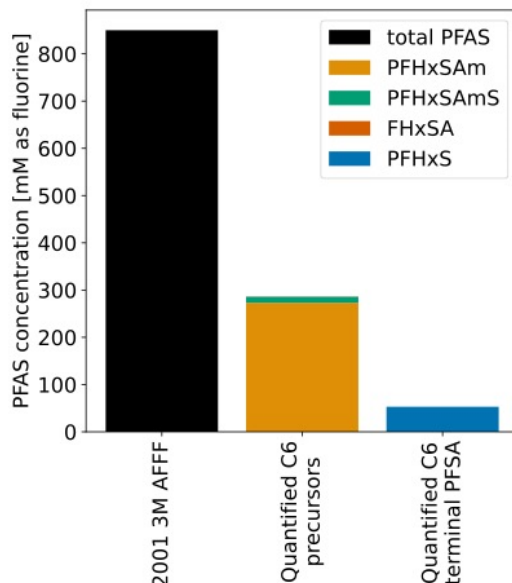


Figure 15. Comparison of C6 PFAS concentrations in a 2001 3M LightWater AFFF.

Concentrations are presented in mM as fluorine. The concentration of total PFAS (black bar) and PFHxS (blue) are presented in Ruyle et al. (2021a).

Active microbial association of PFAS precursors is suggested by the greater loss in the biologically active experiment compared to controls (differences between blue and yellow/green data in **Figure 16** and **Figure 17**).

To quantify rates and magnitudes of sorption processes, we fit a model for time-dependent precursor loss from the aqueous phase (Equation 17) to the experimental data. Equation 17 includes a first-order term for microbial association of precursors (k_{bio} , days⁻¹) and a first-order term for reversible sorption to the sediment/glassware ($k_{control}$, days⁻¹), as follows:

$$\frac{dC}{dt} = k_{control}(C - C(t = \infty)) + k_{bio}C \quad (\text{Equation 17}).$$

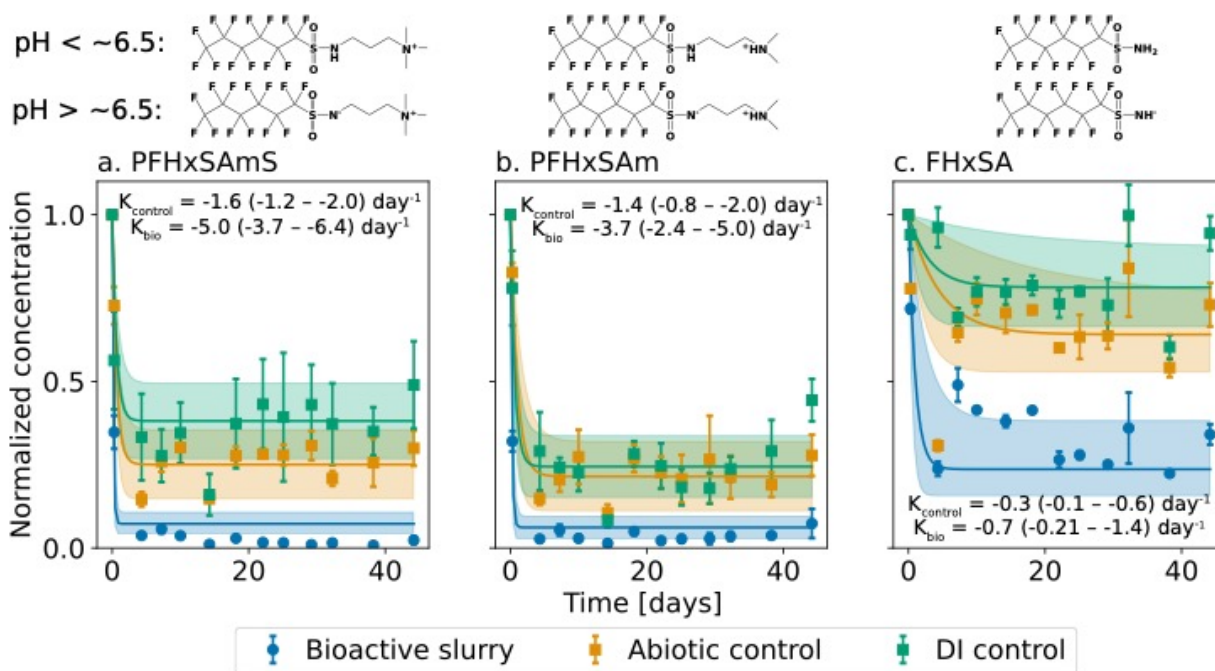


Figure 16. Results of biotransformation experiments at 29°C.

Time-dependent loss of PFAS precursors in a water-sediment slurry incubated at 29°C. Means (circles = bioactive; squares = controls) and standard deviation (error bars) of measurements ($n = 3$ per timepoint) are shown as values normalized to the input concentration. Solid lines show modeled values (expected mean) and shaded regions represent the 90th percentile (%) confidence intervals (CI). Rates (days^{-1} , expected mean and 90th% CI) are shown in text on each panel. The structure of each precursor is shown above the panels including species likely to be abundant in the range of experimental pH.

The rate constant of sediment/glassware sorption and the equilibrium concentration ($C(t=\infty)$, nM) was determined from the control experiments (where there is no microbial activity; Table 25). We used the derived sediment/glassware sorption rate constants in the model for bioactive experiment to derive the rate of microbial association. The expected means of sediment/glassware sorption rate constants ranged between -0.1 and -1.6 days^{-1} .

For all three compounds, the expected means of rate constants for microbial association exceeded sorption by at least a factor of 2x (Table 25) and ranged between -0.2 to -5.0 days^{-1} . At 29°C, the first order half-life of PFHxSAmS and PFHxSAm was less than half a day and approximately 5x faster than the bioassociation of FHxSA (Table 25).

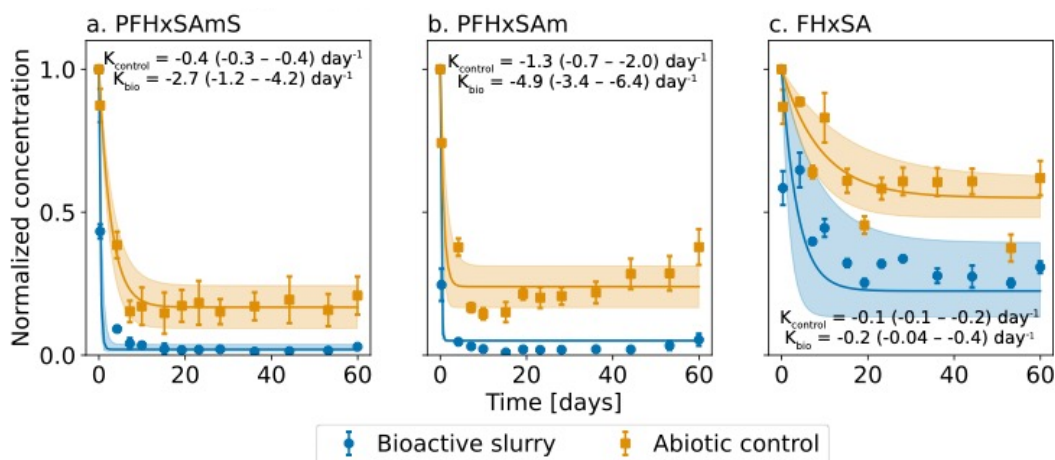


Figure 17. Results of biotransformation experiments at 19°C.

Time-dependent loss of precursors in water-sediment slurry at 19°C. The mean (dots) and standard deviation (error bars) of measurements ($n = 3$ per timepoint) were normalized to the input concentration. Data was modeled according to Equation 17 (shown above the plots) using MCMC. Lines represent the expected mean and shaded regions represent the 90th confidence interval. The expected mean and 90th confidence interval for the rates (in units [days⁻¹]) are shown for each precursor (negative sign indicates a loss process).

We used the SPARC software to predict the speciation of the precursors (ARChem, 2010). The predicted pK_a of the primary amine in all three precursors ranged between 6.2-6.6, indicating that FHxSA likely existed as a mixture between its anionic and neutral species while the zwitterions existed as mixtures between the zwitterionic and cationic species in the microcosms (pH between 6-7.2) (**Figure 16**). The pK_a for the neutralization (cationic $\rightarrow$ neutral) of terminal tertiary/quaternary amine in PFHxSAmS and PFHxSAM was predicted to be greater than 10 and therefore was positively charged throughout the experiments. Positively charged compounds have a greater capacity to associate with the net negatively charged phospholipid bilayer on living cells, which could explain larger losses of PFHxSAmS and PFHxSAM from the aqueous phase compared to FHxSA in the bioactive experiments (**Figure 16**).

Formation of terminal PFHxS is likely microbially limited. Biotransformation of all three precursors was indicated by formation of PFHxS, the terminal product of microbial biotransformation of C6 sulfonamido precursors (**Figure 18**). No formation of PFHxS was observed in the abiotic or Milli-Q water control microcosms.

Formation of PFHxS appeared to follow zero-order kinetics (Equation 18) for all precursors and both experimental temperatures (19°C, 29°C). Specifically:

$$\frac{dC}{dt} = k_{PFHxS} \quad (\text{Equation 18})$$

Table 25. Modeled kinetics of precursor bioassociation and PFHxS formation.^a

		PFHxSA _m S	PFHxSA _m	FHxSA
k_{bio}				
Rate constant [day ⁻¹]	19°C	-2.7 (-1.2 – -4.2)	-4.9 (-3.4 – -6.4)	-0.2 (-0.04 – -0.4)
	29°C	-5.0 (-3.7 – -6.4)	-3.7 (-2.4 – -5.0)	-0.7 (-0.2 – -1.4)
Half-life [day]	19°C	0.3 (0.2 – 0.6)	0.1 (0.1 – 0.2)	3.5 (1.7 – 17)
	29°C	0.1 (0.1 – 0.2)	0.2 (0.1 – 0.3)	1.0 (0.5 – 3.5)
k_{control}				
Rate constant [day ⁻¹]	19°C	-0.4 (-0.3 – -0.4)	-1.3 (-0.7 – -2.0)	-0.1 (-0.1 – -0.2)
	29°C	-1.6 (-1.2 – -2.0)	-1.4 (-0.8 – -2.0)	-0.3 (-0.1 – -0.6)
Half-life [day]	19°C	1.7 (1.7 – 2.3)	0.5 (0.3 – 1.0)	6.9 (3.5 – 6.9)
	29°C	0.4 (0.3 – 0.6)	0.5 (0.3 – 0.9)	2.3 (1.2 – 6.9)
k_{PFHxS}^b				
Rate Constant [nM day ⁻¹]	19°C	6 (1 –12) 10 ⁻³	6 (1 –11) 10 ⁻³	3 (2 –4) 10 ⁻²
	29°C	9 (3 –14) 10 ⁻³	9 (4 –16) 10 ⁻³	8 (6 –9) 10 ⁻²

^aResults from biotransformation experiments in a water-sediment slurry show the expected mean and the 90th confidence interval in parentheses.

^bThe half-life of PFHxS formation is not shown because the zero-order half-life is not dependent on the rate constant.

Formation of PFHxS appeared to proceed at a constant rate throughout the experiment regardless of any changes in the concentrations of reactants (precursors). These results imply that the formation of PFHxS was not limited by the supply of precursors for biotransformation but rather by the microbial reactions catalyzing this transformation. Distributions of data from previously published experimental results showing production of PFOS and PFOA in aerobic soil microcosms from the biotransformation of homologous tertiary and quaternary amine precursors are suggestive of similar pseudo-zero order kinetics (Liu et al., 2021).

Rate constants of PFHxS formation from the zwitterionic precursors ranged from 1 to 16 x 10⁻³ nM day⁻¹. The zwitterionic precursors produced PFHxS at similar rates and displayed minimal temperature dependence (overlapping 90th% CI at 19°C and 29°C) (**Figure 18 a-b**). The PFHxS formation rate from FHxSA was an order of magnitude faster and ranged between 2 to 9 x 10⁻² nM day⁻¹ and more than doubled between the two tested temperatures (**Figure 18c**, Table 25). Thus, despite faster microbial association of the zwitterions compared to FHxSA, the rate limiting step for PFHxS production is the oxidation of the zwitterions to FHxSA rather than oxidation of FHxSA to PFHxS. This could explain why minimal accumulation of FHxSA was observed in the bioactive experiments.

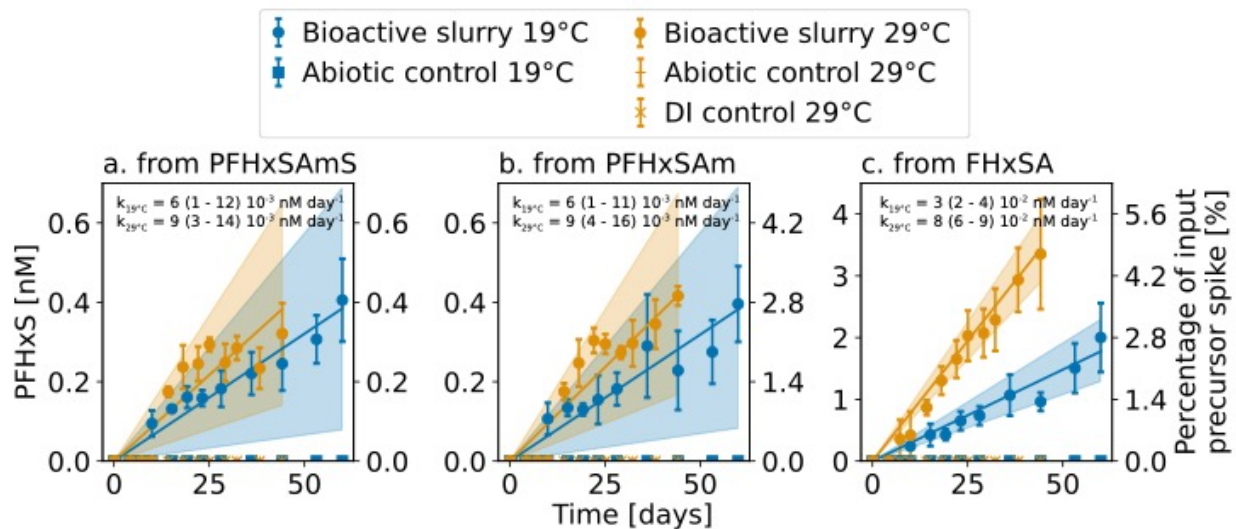


Figure 18. Time-dependent production of PFHxS.

Results show precursor biotransformation in experiments with a water-sediment slurry. Plots show mean concentrations (circles) and standard deviations (error bars) of measurements ($n = 3$ per timepoint) for experiments conducted at 19°C and 29°C. The second y-axis shows the concentration normalized to the input precursor spike. Solid lines show modeled fit (expected mean) and shaded regions denote the 90% CI. Rates (nM day⁻¹, expected mean and 90% CI) are shown at the top of each panel.

HRMS data elucidates potential biotransformation pathway for PFHxSAM. A potential biotransformation pathway for the major C6 precursors in 3M AFFF to PFHxS included six plausible metabolites without commercially available standards (gray, green, and blue structures in **Figure 19**). We detected two of six proposed metabolites in the aqueous phase (perfluorohexane sulfonamido propanoic acid and perfluorohexane sulfinite [PFHxSi]; green structures in Figure 3). The first step of the reaction is microbial association accompanied by nitrification of the terminal tertiary or quaternary amine (we did not detect the secondary or primary amine). The product of this reaction (blue structure in **Figure 19**) could not be monitored because aldehydes require derivatization to stabilize them for detection by ESI-based detection methods. The second step of the potential transformation scheme is oxidation of the aldehyde followed by N-dealkylation of the propanoic acid to FHxSA, while the third and final step of the reaction is nitrification of FHxSA to PFHxSi followed by sulfur oxidation to PFHxS. The reaction pathway includes two nitrification steps (red arrows in **Figure 19**) including the first oxidization of the zwitterions and conversion of FHxSA to PFHxSi.

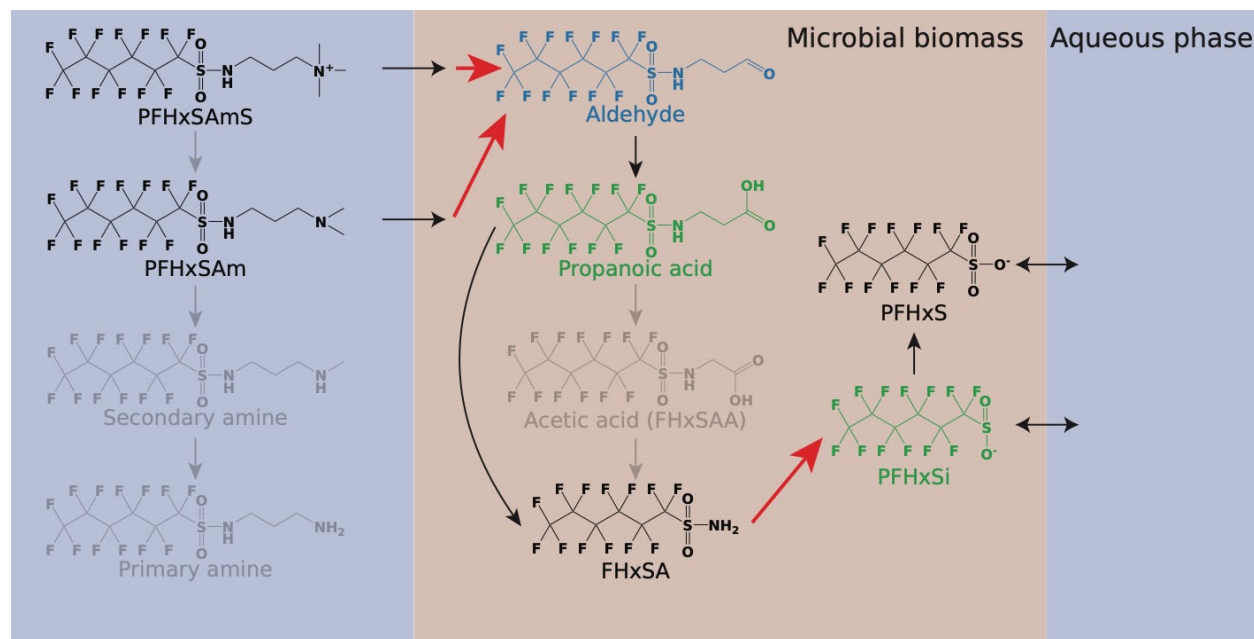


Figure 19. Potential biotransformation pathway of C6 sulfonamido precursors into PFHxS.

Black structures are those with analytical standards. Gray (undetected), green (detected using HRMS), and blue (not detectable using HRMS without derivatization) structures are proposed metabolites without analytical standards. Red arrows indicate reaction steps in which nitrification occurs.

Modeling the temporal evolution of PFHxS formation. We developed a four-compartment box model (**Figure 7**) to better understand biotransformation timescales and production of PFHxS (**Figure 5a,b**). Under aerobic conditions, FHxSA is transformed into PFHxS with only one unstable intermediate (PFHxSi: **Figure 19**), so this is the focus of the modeling. The four compartments include aqueous reservoirs for FHxSA and PFHxS, sorbed (sediment and glassware) FHxSA (sorption of PFHxS is negligible), and biologically associated FHxSA. The biologically associated and sorbed PFHxS reservoirs were negligible (see methods more details) and thus not included in the model. The model was parametrized using experimentally measured rates in Table 25.

Results suggest that most FHxSA was stored in microbial biomass during the experiments (up to 60 days) but only a small fraction of the microbial reservoir was converted to PFHxS (**Figure 20**). Complete (100%) conversion of all the FHxSA to PFHxS was estimated to take 3+ years (**Figure 20c,d**). This may be attributed to non-selective microbial association of precursors, (**Figure 16**) including to 93-99% of non-nitroso/nitrifying microbes that cannot perform the necessary reactions to produce PFHxS (**Figure 19**). In the environment this process would occur over even longer timescales due to lower average annual temperatures. Since the microbiome expands and contracts on faster seasonal timescales, bioassociated precursors could be re-released during colder wintertime months and re-absorbed in warmer months several times before they are ultimately transformed. This is consistent with present-day detections of FHxSA

in Ashumet Pond and further downstream surface waters 20-50 years after AFFF was last used at the military base (Tokranov et al., 2021; Ruyle et al., 2021b).

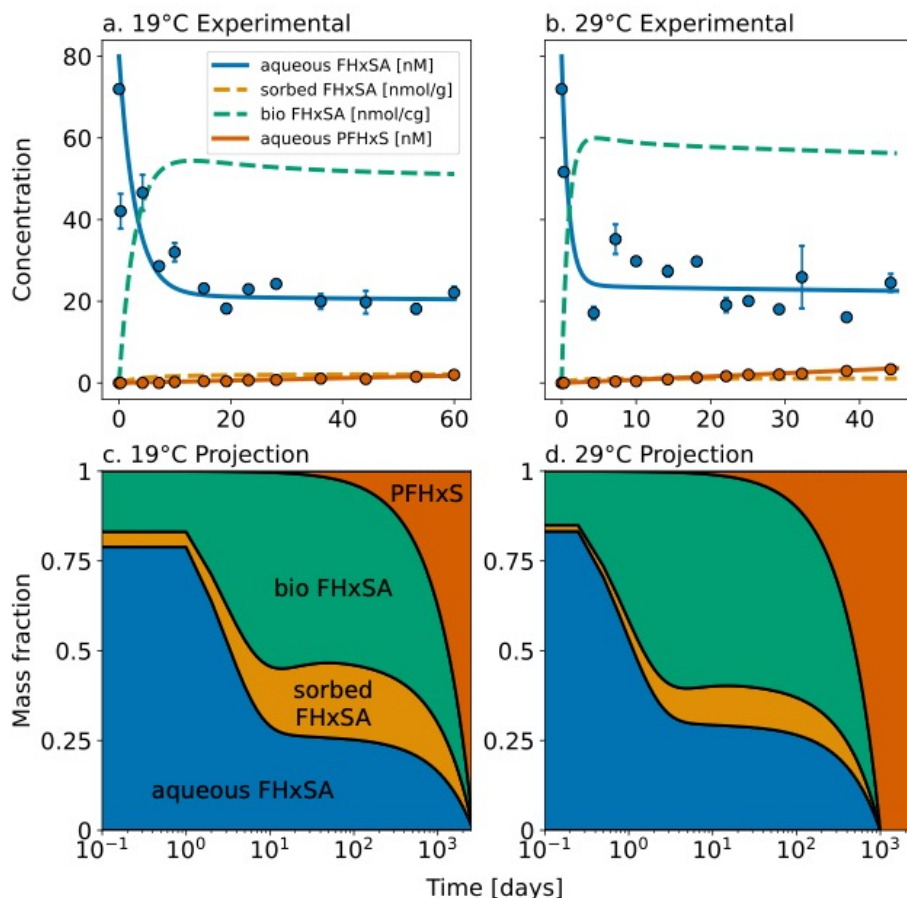


Figure 20. Modeled temporal evolution of precursors at JBCC.

Results from 4-compartment box model parameterized using experimentally measured rates for timescales of FHxSA biotransformation into PFHxS (see Methods). Panels (a) and (b) show the mean (circles) and standard deviation (error bars) of triplicate microcosms. Lines represent the modeled concentrations using the 4-compartment model (solid lines show compartments that were directly measured and dashed lines correspond to model results). We assume the biological mass is 0.1% the sediment mass.²¹ Panels (c) and (d) show the temporal evolution of FHxSA and PFHxS in the aqueous, sorbed, and biological phases.

Experimental and modeling results agree with field observations: The relative stability of precursors against oxidation into PFAA in soils allows for their transport through the vadose zone into groundwater and downstream watersheds (Tokranov et al., 2021). Environmental conditions and microbial communities vary along the flow path, especially near groundwater/surface water boundaries where large gradients in nutrients and redox conditions can be present (Barzen-Hanson et al., 2017, D’Agostino et al., 2017, Houtz et al., 2013, Ruyle et al., 2021b). Past work near the field sampling location for the groundwater/sediment slurries used in this study showed a distinct seasonality in precursor transport across the

groundwater/surface water boundary immediately downgradient from a legacy AFFF plume (Ashumet Pond, MA) (Tokranov et al., 2021). Rapid removal (<36 hours) of up to 85% of precursors was observed in field data during the summer months but not in the winter months.

These field-observations are consistent with the large and rapid (<1 day) bioassociation of precursors observed experimentally in this study (**Figure 16**, **Figure 17**). We estimated based on modeling that biotransformation of all precursors into PFHxS would require more than a year (**Figure 20**). These results suggest that some of the precursors and/or intermediate metabolites associated with the immobile microbiome in the field will be released back into the aqueous phase during winter months when turnover of microbial biomass occurs, which is consistent with prior field observations (Tokranov et al., 2021).

Experimental data from this study appear to show zero-order production of PFHxS (**Figure 18**). Such kinetics suggest microbial limitations on biotransformation rather than the availability of precursors. Theory based on Michaelis-Menten kinetics suggests that zero-order kinetics occurs when the substrate is much more abundant than the Michaelis constant (K_M) or the maximum rate of product formation is much less than the total time of the experiment (poor enzymatic efficiency). Typical values for K_M ($\geq 1 \mu\text{M}$) (Gatto et al., 2019) are orders of magnitude larger than the maximum precursor spike at the beginning of the experiments. This implies PFHxS production could only be enzyme-limited if sulfonamido precursors are in competition with other reduced nitrogen species for coordination sites. In the bioactive experiments, zero-order production of nitrate exceeded what could be potentially released upon complete biotransformation of the spiked precursor pool (**Figure 21**). Future work should identify specific enzymes responsible for biotransformation to understand the role of competing substrates and reaction efficiency on precursor reaction rates.

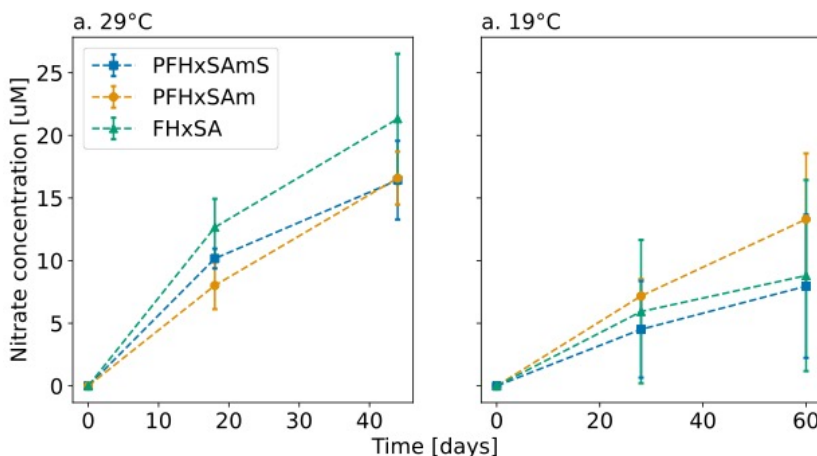


Figure 21. Concentration of nitrate in bioactive experiments.

Dots represent the mean and error bars represent the standard deviation from three replicate bottles. Dashed lines connect the temporal data. Panel a shows data from the experiment conducted at 29°C corresponding to PFAS data presented in **Figure 16** and **Figure 18**. Panel b shows data from the experiment conducted at 19°C corresponding to PFAS data presented in **Figure 17** and **Figure 18**.

Task 3 Results and Discussion: JBCC fire-training area model

In groundwater samples obtained from below the former fire-training area at JBCC, precursors accounted for $46 \pm 8\%$ of total PFAS (Figure 22). The relative abundance of ECF-derived precursors (**Error! Reference source not found., Table 12**) in groundwater suggests that the use of 3M products constituted approximately 2:1 ratio of total AFFF use at the site, confirming results from the Quashnet River. High resolution mass spectrometry yielded unambiguous spectrum-structure matches for 15 precursors, 9/15 of which were C4 and C6 perfluoroalkyl sulfonamido compounds that have either been previously identified in 3M AFFF or are expected biodegradation intermediates (Table 26). None of the identified precursors originating from 3M AFFF are included in current standard U.S. Environmental Protection Agency (U.S. EPA) PFAS methods. We used extractable organofluorine (EOF) measurements as a proxy for total PFAS in groundwater below the fire training area at JBCC. All EOF ($101 \pm 7\%$) was accounted for by combining targeted PFAS measurements with the TOP assay interpreted using Bayesian inference (Figure 22). The C6 and C8 PFSA and the C7 PFCA were the predominant terminal PFAS, consistent with their composition in ECF AFFF (Ruyle et al. 2021a; Houtz et al. 2013).

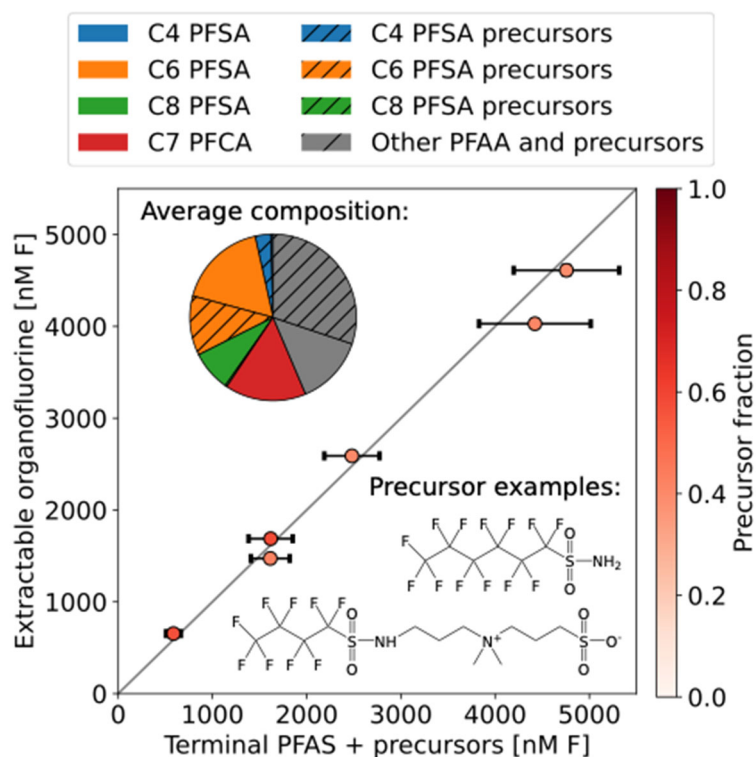
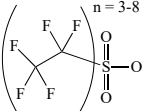
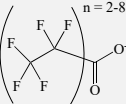
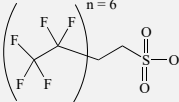
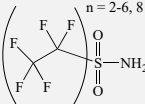
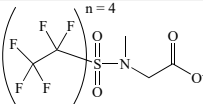
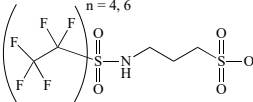
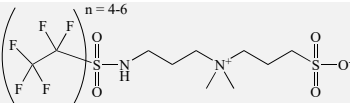
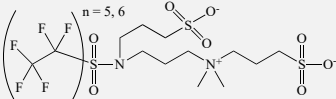


Figure 22. Comparison of extractable organofluorine (EOF) and quantified PFAS in groundwater at the former fire training area.

The x-axis is the sum of terminal PFAS measured in extracts split between EOF and LCMS and oxidizable precursors inferred from the TOP assay. X-error bars represent the sum of the percent difference in terminal PFAS from duplicate extracts (9%; $n = 1$) and the standard deviation of the posterior estimate of

precursors. Y-error bars represent the percent difference in EOF from duplicate extracts (1%; n = 1).
Data is shaded by the fraction of explained EOF and compared to the one-to-one line (gray).

Table 26. Summary of PFAS identified by suspect screening.

PFAS	Registered CAS number	Confidence level ¹	ESI mode	Structure
<i>Perfluoroalkyl sulfonates (PFSA)</i>				
PFPrS	423-41-6	2a	—	
PFBS	375-73-5	1	—	
PFPeS	2706-91-4	1	—	
PFHxS	355-46-4	1	—	
PFHpS	375-92-8	1	—	
PFOS	1763-23-1	1	—	
<i>Perfluoroalkyl carboxylates (PFCA)</i>				
PFPrA	422-64-0	2a	—	
PFBA	375-22-4	1	—	
PFPeA	2706-90-3	1	—	
PFHxA	307-24-4	1	—	
PFHpA	375-85-9	1	—	
PFOA	355-67-1	1	—	
PFNA	375-95-1	1	—	
<i>n:2 fluorotelomer sulfonates (n:2 FTSA)</i>				
6:2 FTSA	27619-97-2	1	—	
<i>Perfluoroalkyl sulfonamides (FASA)</i>				
FEtSA	78491-70-0	2a	—	
FPrSA	NA	2a	—	
FBSA	30334-69-1	1	—	
FPeSA	82765-76-2	2a	—	
FHxSA	41997-13-1	1	—	
FOSA	754-91-6	1	—	
<i>Perfluoroalkyl sulfonamido acetic acids (FASAA)</i>				
N-MeFBSAA	159381-10-9	2a	—	
<i>Class 1 perfluoroalkyl sulfonamido precursors (N-SP-FASA)²</i>				
N-SP-FBSA	2089108-62-1	2a	—	
N-SP-FHxSA	2089108-64-3	2a	—	
<i>Class 2 perfluoroalkyl sulfonamido precursors (N-SPAmP-FASA)²</i>				
N-SPAmP-FBSA	2089108-66-5	2a	—/+	
N-SPAmP-FPeSA	2089108-67-6	2a	—/+	
N-SPAmP-FHxSA	2089108-68-7	2a	—/+	
<i>Class 5 perfluoroalkyl sulfonamido precursors (N-SPAmP-FASAPS)²</i>				
N-SPAmP-FPeSAPS	2089108-79-0	2a	—/+	
N-SPAmP-FPHxSAPS	2089108-80-3	2a	—/+	

¹1 = confirmed structure; 2a = probable structure matched to reference MS/MS spectrum (according to Schymanski et al., 2014); ²Nomenclature based on Barzen-Hansen et al. (2017)

Biodegradation of C4 and C6 sulfonamido precursors produces PFSA with the same number of perfluorinated carbons (Choi et al. 2022). In subsurface environments, where direct and indirect photolysis cannot occur, this is the only mechanism of precursor degradation. We can compare the measured ratios of precursors and PFSA to their terminal PFSA to understand the potential for additional production from degradation. The ratio of precursors to terminal PFSA in AFFF and groundwater is greatest for the C4 compounds (AFFF = 10.9 ± 4.7 ; groundwater = 4.9 ± 1.6), intermediate for the C6 compounds (AFFF = 6.7 ± 1.4 ; groundwater = 0.7 ± 0.4), and lowest for the C8 compounds (AFFF = 0.03 ± 0.00 ; groundwater = 0.04 ± 0.01). The ratio of precursors to their terminal PFSA decreased significantly for all chain lengths except C8 between 3M AFFF and groundwater (ANOVA type III; $f > 10$, $p < 0.001$). This is consistent with slower transport of precursors and/or biodegradation into terminal compounds in the environment following AFFF use.

Figure 23 shows large temporal fluctuations in PFAS concentrations but no consistent temporal trends. Analytical precision and interlaboratory variability between our lab and the U.S. National Institute of Standards and Technology was $< 20\%$ (except for interlaboratory variability in the C6 PFSA = 35%). Highest PFAS concentrations were observed in both the earliest (2007) and penultimate (2020) samples. Lowest PFAS concentrations were observed in 2021 one year after the maxima for several compounds in 2020. Samples collected in June and July of 2014 and July and November 2015 (**Table 9**) show large variability in the C6 and C8 PFSA concentrations (20–43%). Together, these data suggest natural attenuation of the PFAS contamination in the fire-training area through transport and/or degradation has not been significant over the monitoring period between 2007 and 2021.

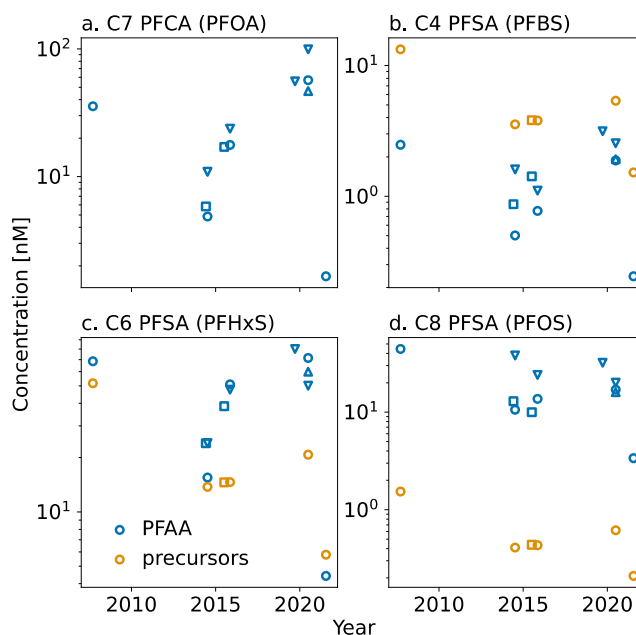


Figure 23. Temporal variability in groundwater PFAS at JBCC.

Measured variability in PFAS concentrations in groundwater beneath JBCC former fire-training area between 2007-2021 ($n = 12$ time intervals sampled). Concentrations of terminal PFAS are shown in blue and their precursors are shown in yellow. Data reported in this study (circles), Weber et al. (squares), the Air Force Civil Engineer Center (downward triangle), and the National Institute of Standards and Technology (upward triangle) are distinguished by different markers. Dashed lines connect sequential data reported in this study.

We hypothesized that observed temporal differences in groundwater PFAS concentrations reflect dynamic soil saturation conditions. Maximum hydraulic conductivity in the sandy/gravel sediment found on Cape Cod, MA occurs when soils are saturated, increasing advective transport of the most water soluble PFAS. Retardation of PFAS transport in groundwater occurs when they sorb to the surface of soil/sediment solids and/or are retained at the air-water interface (Brusseau et al. 2018; Guo et al. 2022). Saturation leads to the collapse of the air-water interface, allowing surface-active PFAS to migrate into the aqueous phase (Brusseau et al. 2018; Guo et al. 2020).

To test this hypothesis, we examined county level data on the Drought Severity and Coverage Index (DSCI) over time. To examine more specific site conditions, we used the elevation of a nearby kettle lake (Ashumet Pond) as a proxy for soil saturation. The groundwater-fed lake height is controlled by the position of the groundwater table, which itself responds to the balance of precipitation and evapotranspiration mediated by soil saturation on timescales of weeks to months (Massey et al. 2006). We therefore interpret elevation of pond height to be the result of recent precipitation and high soil moisture conditions.

Moderate drought conditions at the field site in July 2021 corresponded the lowest concentrations of the 4 terminal PFAS with in groundwater sample as well as precursors (**Figure 23**). Increasing DSCI indicative of the onset of drought conditions ($DSCI > 0$) began in May 2021 and continued to rise until the sampling date ($DSCI = 123$). DSCI indicative of abnormally dry conditions increased from 13 to 64 between June and July 2014 when we observed a decline in up to 18–54% in terminal PFAS between months (**Figure 23**). These data suggest dynamic soil moisture conditions in the vadose zone substantially influence groundwater PFAS concentrations.

We found statistically significant correlations between groundwater PFAS concentrations and the height of the kettle lake for the C7 PFCA and C4 PFSA (p -value < 0.05 ; **Figure 24**). Increased groundwater concentrations of these PFAS were observed when lake height and soil moisture were high, consistent with our hypothesis. A positive but non-significant relationship was observed between concentrations of the C6 PFSA and lake height. No relationship was apparent between lake height and the C8 PFSA and PFSA precursors of all chain lengths (**Figure 24, Figure 25**) suggesting processes other than soil moisture such as soil/sediment sorption (Brusseau et al. 2018) or nonideal transport phenomena (Brusseau et al. 2019) contribute to the temporal patterns for these compounds.

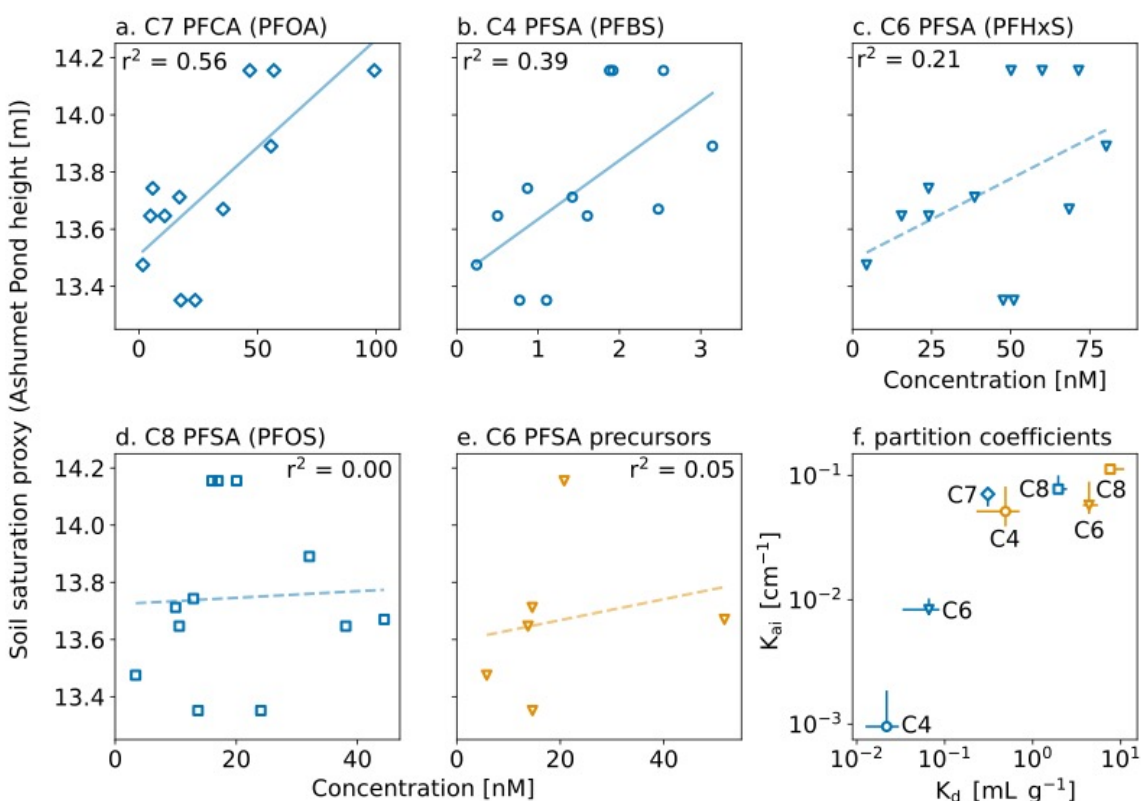


Figure 24. Relationship between PFAS concentrations and a proxy for soil saturation.

Panels a-e shows the relationship between PFAS concentrations and the height of Ashmet Pond on the same day ($n = 12$ for terminal PFAS in blue; $n = 6$ for precursors in yellow). Plots for C4 and C8 PFSA precursors are shown in **Figure 25**. Lines represent the best fit ordinary least squares linear relationship (solid = p -value < 0.05 ; dashed = p -value > 0.05). Panel f shows site-specific sediment-water ($\log K_d$) and air-water interface ($\log K_{ai}$) partition coefficients for terminal PFAS and precursors. Error bars represent the interquartile range.

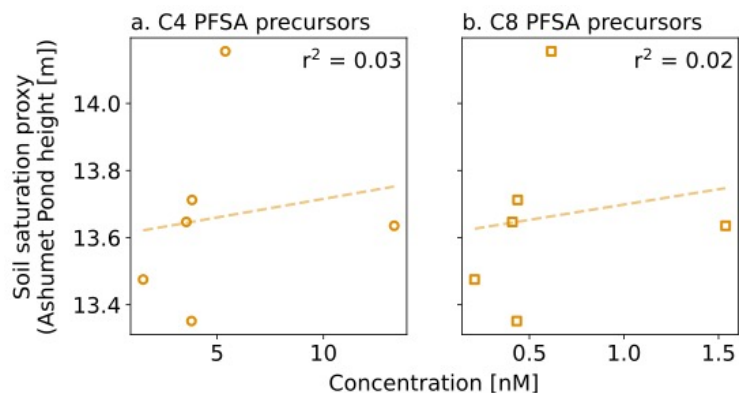


Figure 25. Relationship between PFAS concentrations and a proxy for soil saturation continued.

Panel shows the relationship between precursor concentrations and the height of Ashumet Pond on the same day ($n = 6$). Plots for terminal PFAS and C6 PFSA precursors are shown in **Figure 24**. Lines represent the best fit ordinary least squares linear relationship (solid = p -value < 0.05 ; dashed = p -value > 0.05).

Cumulative releases of the four main terminal PFAS in 3M AFFF (C7 PFCA, C4, C6, and C8 PFSA) and their precursors were estimated to be 350 mol (interquartile range [IQR] = 270-540 mol) from the Bayesian inference. Constraints from measured groundwater PFAS concentrations over time suggest more than 99% of PFAS were released between 1970-1985, when the site was actively used as a fire training area. Direct measurements presented in this study thus reflect the conditions of the plume 21 to 55 years after most AFFF release. Model results show soil is the primary reservoir for PFAS except for the C4 PFSA (**Figure 26**). Soil reservoirs exceed groundwater reservoirs by a factor of 2-22 between 2007 and 2021. These results agree with meta-analyses of contaminated sites from around the world, including those where AFFF was used, that typically show higher PFAS concentrations in soil compared to underlying groundwater (Anderson et al. 2019; Brusseau et al. 2020).

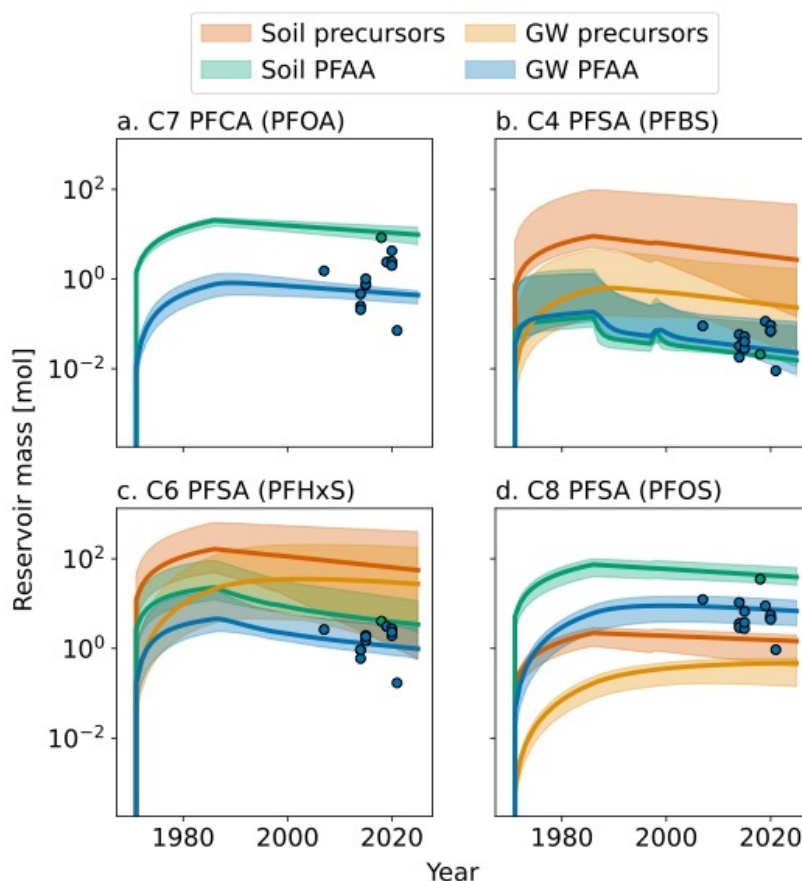


Figure 26. Modeled PFAS reservoirs at JBCC.

Optimized model reservoirs of PFAS beneath the former fire training area. Shaded regions represent the modeled range of expected reservoirs estimated as the minimum and maximum of 100 random samples from posterior models. The solid line represents the maximum a posteriori estimation. Dots represent measurements. Results are shaded according to their respective reservoir (soil versus groundwater

[GW]) and chemical speciation (precursor versus PFAA) between 1970 (the first use of the fire training area) and 2025.

Variable retention of PFAS in soil (**Table 27**) agree with expected physiochemical differences due to chain length and head group chemistry. For PFSA, soil residence times increase significantly with perfluorinated chain length (**Table 27**; Type I ANOVA; f -value = 6×10^4 , p -value < 0.01) as compounds become increasingly hydrophobic, thus increasing their K_d and K_{ai} . The residence time of PFSA precursors in soil also increased with chain length and were significantly greater than their terminal PFSA (ANOVA type III; f -values > 1E3, p -values < 0.0001) also due to both greater K_d and K_{ai} (**Figure 24**). Larger precursor K_d s can be attributed to the lack of electrostatic repulsion between neutral and cationic functional groups and net negatively charged soil/sediment surfaces at the site. Larger precursor K_{ai} s is consistent with original patent applications that describe greater surface activity compared to PFSA for precursors with quaternary amine functional groups such as those found at the site (e.g., **Figure 22**; **Table 26**) (Bertocchio et al. 1991).

Table 27. Inferred* PFAS transport and biodegradation parameters.

	<u>Precursors</u>		<u>PFAA</u>		<u>Biodegradation</u>	
	R _{Soil}	R _{GW}	R _{Soil}	R _{GW}	k [yr ⁻¹]	t _{1/2} [yr]
C7 PFCA	—	—	531 (425-598)	2 (2-2)	—	—
C4 PFSA	385 (290-604)	3 (2-4)	8 (8-15)	1 (1-1)	0.007 (0.005-0.013)	104 (52-136)
C6 PFSA	490 (418-748)	19 (17-24)	64 (58-78)	1 (1-1)	0.008 (0.005-0.020)	90 (35-129)
C8 PFSA	932 (850-1007)	33 (32-47)	602 (597-780)	9 (8-11)	0.0001 (0.0002-0.005)	5389 (139-4087)

*Reported values represent the joint maximum a posteriori (MAP) estimation and the marginal interquartile range (in parentheses) for individual PFAA and PFSA precursors. Retardation coefficients (R) are with respect to water transport time through soil (0.1 yr) and groundwater (GW) (1 yr) and are unitless. Biodegradation rates of precursors into PFSA are assumed to be first-order.

The significantly different retention of the C8 PFSA versus the C7 PFCA (two-sided t-test; t -value = 38, p -value < 0.001) is due to differences in K_d rather than K_{ai} (**Figure 24**). This finding deviates from controlled laboratory experiments that found greater surface activity of the C8 PFSA compared to the C7 PFCA (Campbell et al. 2009). Although we have shown excellent agreement among the fate of PFAS reported here and physiochemical properties reported in the experimental/mathematical literature, this discrepancy highlights the importance of robust field data in evaluating the relevance of prior work because many additional covariates (pH, ionic strength, co-solvents) can impact environmental behavior.

The smaller residence times of the C4 PFSA (2 yr) and C6 PFSA (7 yr) suggest they should be rapidly attenuated and minimally abundant during the sampling campaign which corresponds to 3 – 28 residence times after the majority of AFFF release. However, elevated concentrations of both PFAS persist in groundwater at the site where the C6 PFSA is the most

abundant terminal compound in every sample (**Figure 23**). We hypothesize that biodegradation of more sorptive and surface-active precursors sustains elevated concentrations of the C4 and C6 PFSA. Using the model, we first estimated a first-order precursor biodegradation rate of $0.007\text{--}0.008\text{ yr}^{-1}$ (IQR: $0.005\text{--}0.02\text{ yr}^{-1}$) from the data optimized solution (**Table 27**). We then determined the contribution of precursors to observed PFSA concentrations by rerunning the optimized model without any precursor inputs and calculated the percent decrease in PFSA groundwater reservoirs over time. Results suggest that approximately 100% of the observed C4 PFSA and the majority of the C6 PFSA are attributed to precursor biodegradation (**Figure 27**). This result is supported by high resolution mass spectrometry, which identified several C4 and C6 perfluoroalkyl sulfonamido precursors that are expected biodegradation intermediates in between precursors that originate from 3M AFFF and terminal PFSA (**Table 26**). Conversely, precursor biodegradation is less constrained and has minimal impact on the C8 PFSA reservoir due to low relative abundance of C8 PFSA precursors in 3M AFFF.

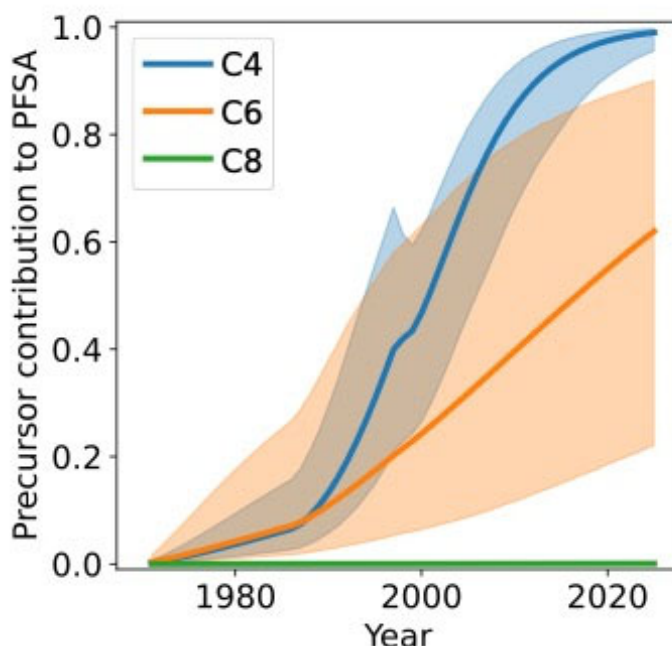


Figure 27. Modeled contributions of precursor degradation to terminal PFAS in groundwater.

Modeled contributions of C4, C6, and C8 precursor degradation to PFBS, PFHxS, and PFOS in groundwater. Lines represent one minus the quotient of PFSA groundwater reservoirs between 1970-2021 in model simulations without and with precursor emissions using MAP parameter estimates. Shaded regions represent the bounds from 1000 random simulations from the posterior.

Task 4 Results and Discussion: Passive samplers

Uptake of PFAS by PE tube samplers in laboratory experiments. Uptake of PFAA by the novel tube passive sampler in the laboratory experiments increased with chain length. Longer-chain compounds PFOA ($100 \pm 7 \text{ mL day}^{-1}$) and PFOS ($400 \pm 200 \text{ mL day}^{-1}$) showed larger mean uptake than the model compounds PFHxA ($90 \pm 8 \text{ mL day}^{-1}$) and PFHxS ($100 \pm 100 \text{ mL day}^{-1}$) at flow rates $10\text{--}60 \text{ cm s}^{-1}$ and 15°C . The uptake of the shorter chain length PFBS ($40 \pm 20 \text{ mL day}^{-1}$) and PFPeA ($20 \pm 0.5 \text{ mL day}^{-1}$) was lower than for longer chain compounds (**Table 28**).

Table 28. Laboratory and field observed sampling rates.

Compound	Temp $^\circ\text{C}$	Lab Observed R_s (mL day^{-1}) $n = 3$	Field Observed R_s (mL day^{-1}) $n = 11$	3-Phase Model R_s (mL day^{-1})	2-Phase Model R_s (mL day^{-1})
PFBA	15	20 (± 20)	20 (± 06)	200 (± 100)	20 (± 0.2)
	25	10 (± 70)		300 (± 100)	20 (± 0.2)
PFBS	15	40 (± 20)	50 (± 20)	200 (± 100)	20 (± 0.4)
	25	100 (± 70)		300 (± 200)	20 (± 0.3)
PFPeA	15	50 (± 40)	30 (± 10)	70 (± 30)	40 (± 0.1)
	25	50 (± 10)		100 (± 40)	40 (± 0.1)
PFHxA	15	100 (± 80)	40 (± 10)	60 (± 30)	20 (± 0.4)
	25	100 (± 50)		70 (± 0.40)	20 (± 0.3)
PFHxS	15	100 (± 100)	50 (± 20)	100 (± 40)	20 (± 0.5)
	25	200 (± 70)		100 (± 60)	20 (± 0.3)
PFHpA	15	70 (± 60)	50 (± 20)	200 (± 70)	30 (± 0.9)
	25	200 (± 100)		200 (± 100)	30 (± 0.7)
PFHpS	15	100 (± 100)	70 (± 60)	300 (± 100)	20 (± 0.6)
	25	300 (± 100)		300 (± 100)	20 (± 0.5)
PFOA	15	100 (± 20)	50 (± 20)	50 (± 40)	20 (± 0.4)
	25	100 (± 60)		60 (± 50)	20 (± 0.3)
PFOS	15	400 (± 200)	50 (± 20)	500 (± 200)	10 (± 0.3)
	25	300 (± 200)		600 (± 200)	10 (± 0.3)

Data includes 9 PFAA compounds of interest compared to 2- and 3- phase model predicted sampling rates at two temperatures for flow rates between $0.5\text{--}60 \text{ cm/s}$. All observed values display one standard deviation, all predicted models display the uncertainty associated with the predicted value in the same units.

Increasing uptake with increasing chain length was also generally exhibited by the predicted sampling rates generated using the 3-phase model (**Table 28**). An exception was PFBS, which had an anomalously high predicted sampling rate ($400 \pm 8 \text{ mL day}^{-1}$) probably due to uncertainty in sorbent or membrane partitioning values derived in the laboratory.

Influence of water flow velocity on R_s . For PFHxA and PFHxS, uptake rates were generally not a function of flow rate between $10\text{--}60 \text{ cm s}^{-1}$ for laboratory, field, or 3-phase model observed sampling rates (**Figure 28**). This trend was further observed across all compounds in **Table 28**. The lack of water flow effect on sampling rate extended down to 0.5 cm s^{-1} based on the model

(Equation 2) for flow rates below the 10 cm s⁻¹ limit of most handheld flow meters. A lower value of approximately 0.5 cm s⁻¹ flow speed is predicted to result in similar sampling rates as those seen between 10-60 cm s⁻¹ (**Figure 29, Figure 30**)

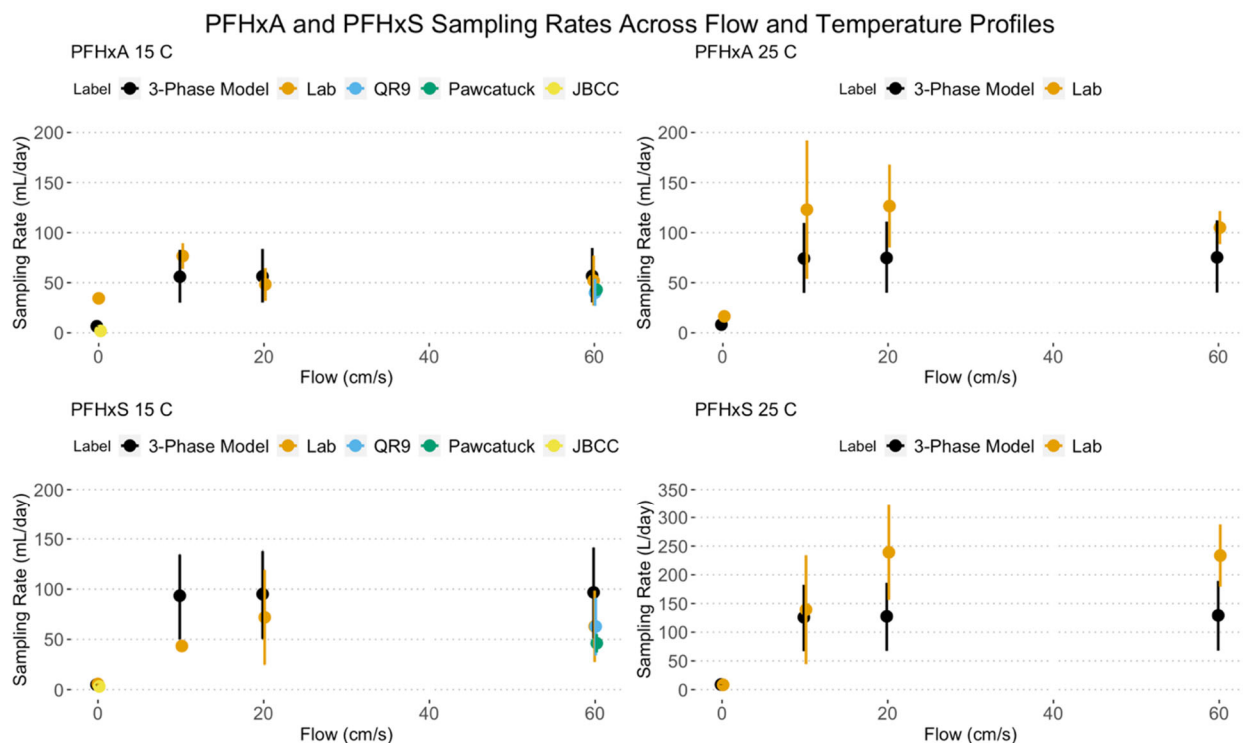


Figure 28. Modeled and observed sampling rates of PFHxA and PFHxS (L/day).

Results show two temperatures in the field deployments (15 °C) and laboratory experiments (15 and 25 °C). Error bars display 1 standard deviation for measurements. Modeled data displays the uncertainty of the predicted sampling rate based on the standard deviation amongst replicate samples used to calculate K_{mw} .

PFHxA and PFHxS Sampling Rate At Low Velocities (<10 cm/s) at 15 C

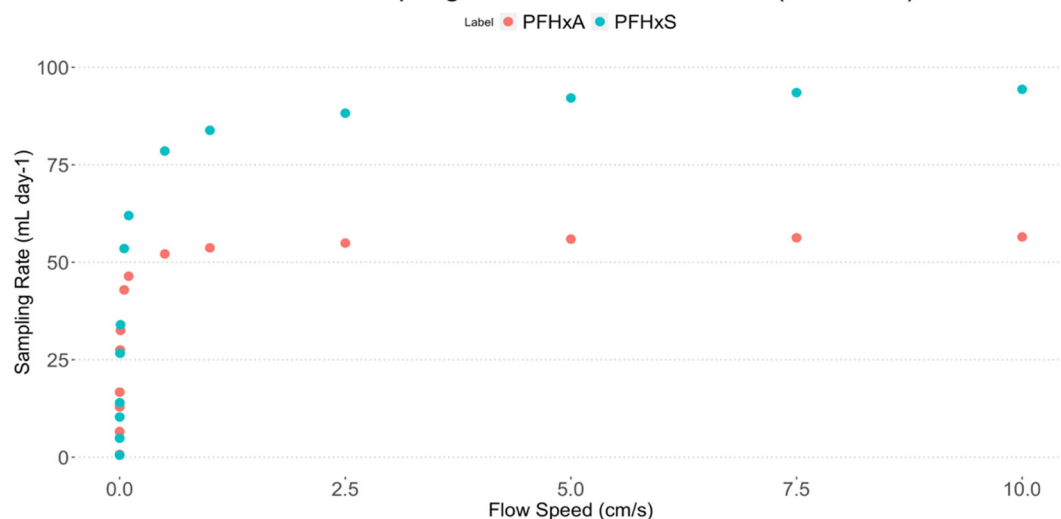


Figure 29. Modeled sampling rates of low flow conditions.

Flow conditions range from 1.0×10^{-6} cm s⁻¹ to 10 cm s⁻¹ for both PFHxA and PFHxS.

PFHxA Sampling Rate At Low Velocity Speeds <10 cm/s and 15 C

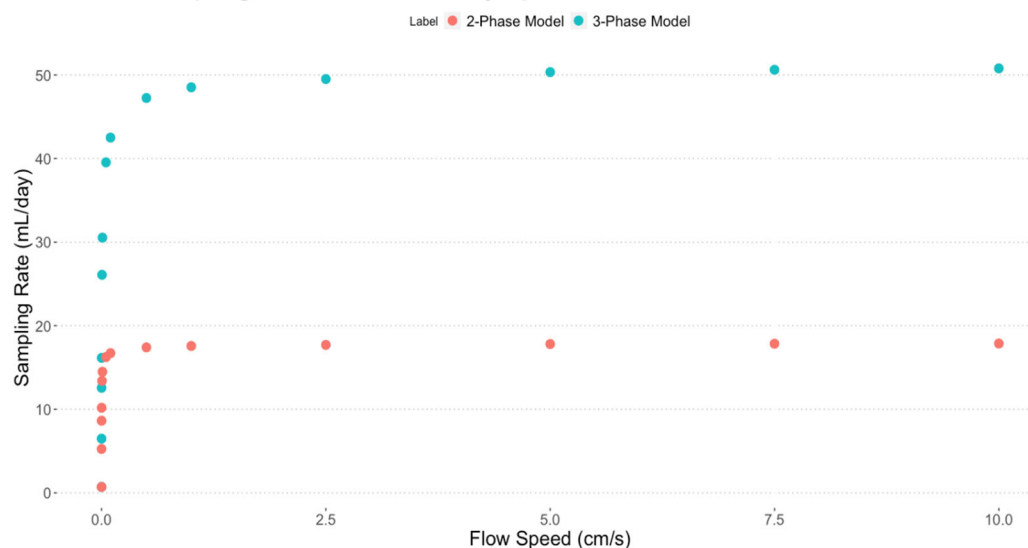


Figure 30. Modeled sampling rates of low flow conditions part II.

Flow conditions range from 1.0×10^{-6} cm/s to 10 cm/s for both the two phase and three phase modeling approaches suggested for investigation. This comparison suggests that the resistance to flow impacts is not impacted by partitioning or sorbent choice, which the two-phase model neglects.

Influence of temperature on R_s . The laboratory observed sampling rates showed an increase in PFHxA uptake with a 10 °C increase in temperature. The tube passive sampler also took up PFHxAs at higher sampling rates than PFHxSs of similar chain length (Table 28, Figure 28, Figure 29). Across the two temperature profiles, the 25 °C laboratory observations exhibited a larger

standard deviation in sampling rate than what as observed in the 15 °C or field observed sampling rates.

Uptake of PFAS by PE tube. The uptake of PFAAs by the empty PE tube walls increased with chain length, in agreement with the trend exhibited by the passive sampler deployments (**Figure 31,**

Table 29). PFSAAs exhibited higher partitioning rates between membrane-water phases than their PFCA homologues (**Figure 31,**

Table 29). There was little difference between the K_{mw} values for PFAA compounds at 15 and 25°C so average values of all replicates were used to determine a single K_{mw} value per compound. Published values for PFAA partitioning to PE was limited, though a published value for PFOS was within 20% of the PFOS K_{mw} reported in this research n (Wang et al., 2015). The average standard deviation amongst compounds was 48%, which was used to calculate the uncertainty of the model predicted value and the standard deviation error bars on **Figure 31**.

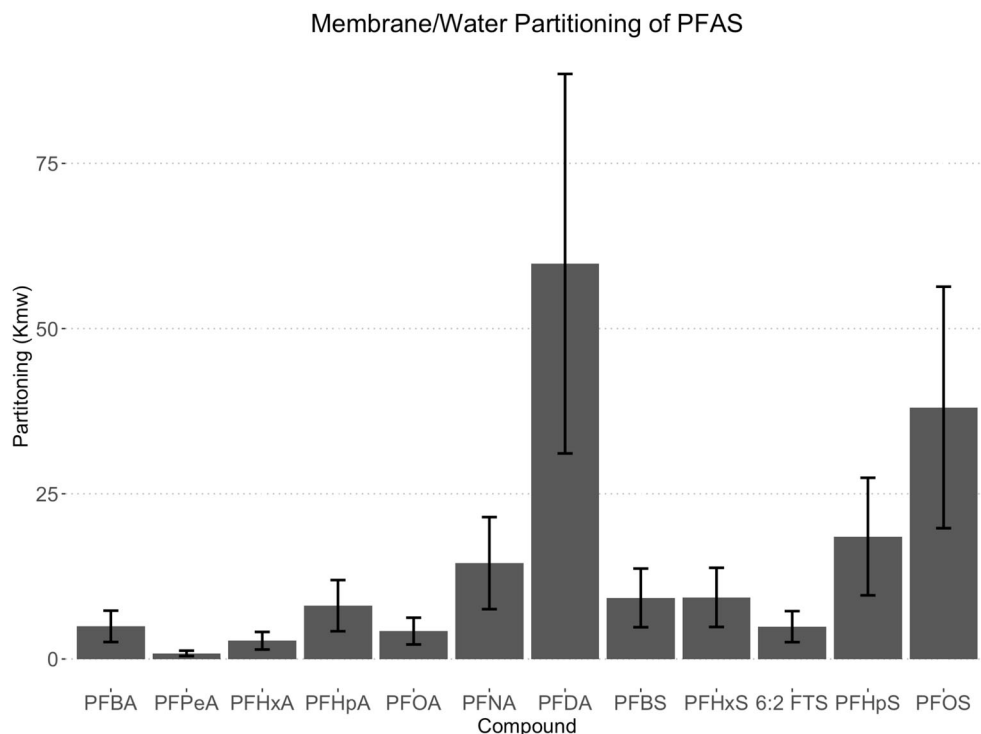


Figure 31. Mean partition coefficients of PFAS between the microporous membrane and water.

Error bars indicate average standard deviation.

Table 29. Sorbent-water (K_{sw}) and membrane-water (K_{mw}) partition coefficients.

Compound	Log K_{sw} ($\pm 3.4\%$) n = 3	K_{mw} ($\pm 48\%$) n = 9
PFBA	2.7 (± 0.09)	4.9 (± 2.3)
PFPeA	3.8 (± 0.13)	0.86 (± 0.4)
PFHxA	4.8 (± 0.16)	2.8 (± 1.3)
PFHpA	4.6 (± 0.16)	8.1 (± 3.9)
PFOA	4.6 (± 0.16)	4.2 (± 2.0)
PFNA	6.0 (± 0.20)	15 (± 7.2)
PFDA	4.9 (± 0.17)	60 (± 29)
PFBS	4.8 (± 0.16)	9.2 (± 4.4)
PFHxS	4.2 (± 0.14)	9.3 (± 4.4)
PFHpS	5.2 (± 0.18)	19 (± 9.1)
PFOS	5.6 (± 0.19)	38 (± 18)
6:2 FTSA	3.1 (± 0.15)	4.9 (2.4)

**Values derived in laboratory batch experiments for use in the modeling of passive sampler uptake for 9 PFAA compounds plus three additional PFAS compounds of interest. K_{sw} values for HLB sorbent-water are dimensionless and reported as Log K_{sw} . K_{mw} values are also dimensionless, but not reported in Log format due to lower binding affinity of PFAS for the high density polyethylene. Both K terms are reported with average percent standard deviation across each batch.*

Uptake of PFAS by sorbent. Uptake of PFAS by the HLB sorbent generally increased with chain length (Table S4) for PFCAs and PFSAs. Log K_{sw} ranged from 2.7-6.0 from the C4 PFBA to C9 PFNA. PFSAs displayed a greater log K_{sw} values: 4.8 for PFBS, and a linear increase from PFHxS to PFOS (Log K_{sw} 4.2-5.6). Sorption to HLB was consistent, with an average standard deviation of 3.4%, suggesting that the uptake of PFAS by the HLB sorbent is not a major source of uncertainty in the model derived sampling rate predictions.

Uptake of PFAS by bio fouled tubes. To validate the performance of these passive samplers in field conditions, biofouling might introduce an additional resistance to uptake. There was no visible difference between the mass of PFAS on clean and bio fouled tubes during concurrent laboratory experiments (**Figure 32**). This can be explained by considering how thin a layer of algae, sponge, or other organic matter (**Figure 33**) compares to the thick polyethylene membrane in thickness and permeability. The high-water content of any algae or sponge species inhabiting the membrane would suggest low impact on the aqueous diffusivity or partitioning of a given PFAS compound.

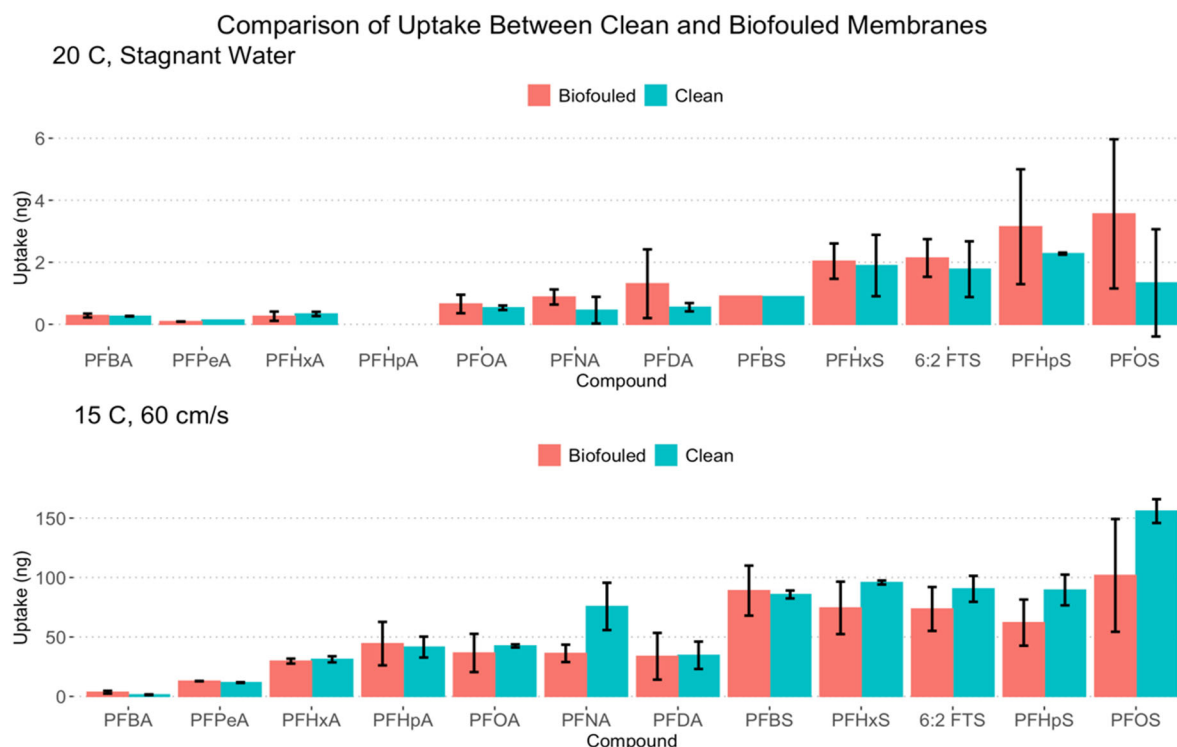


Figure 32. Results for co-deployed tube samplers.

Comparison of mean PFAS mass (ng) measured in co-deployed tube samplers under similar conditions in the experimental tank (error bars represent one standard deviation).

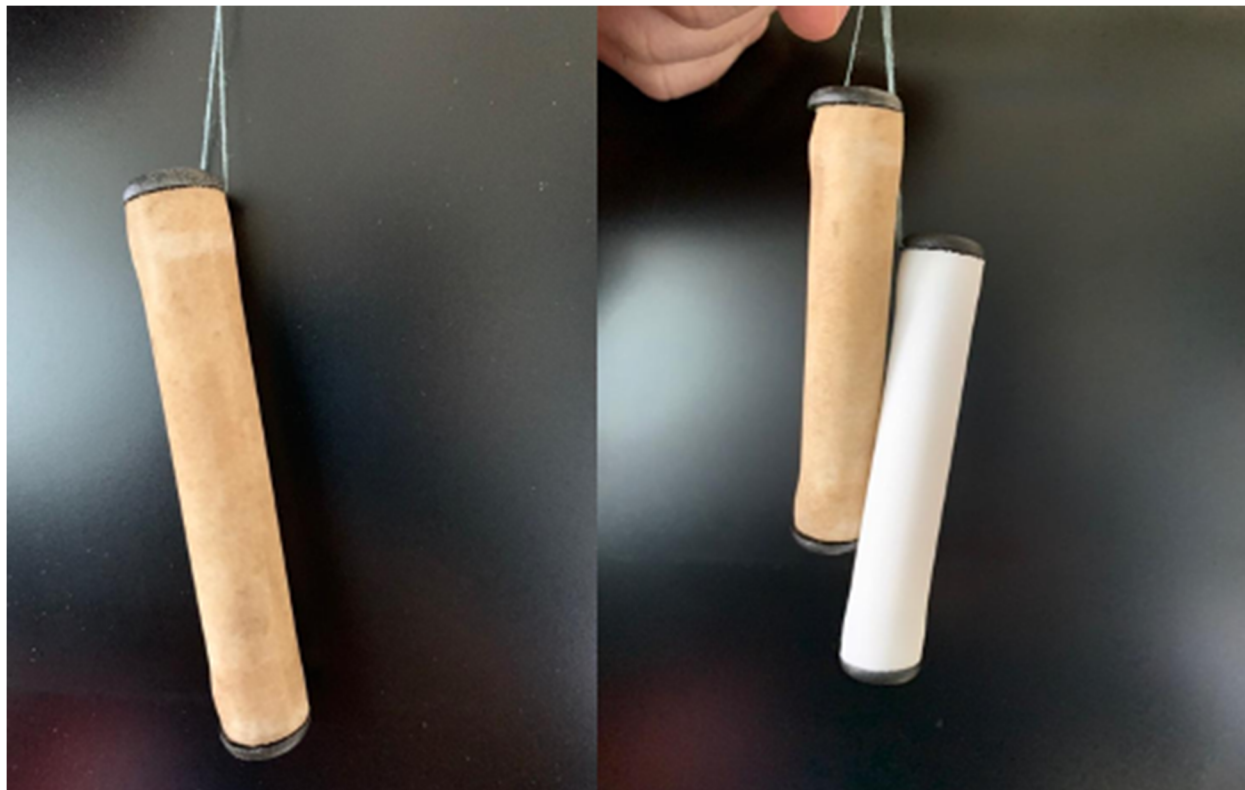


Figure 33. Comparison of biofouled and clean tube sampler.

Example of a biofouled tube sampler after one month in the field compared to a clean, laboratory deployed tube sampler. The “Empty” phase refers to the sampling rate of the polyethylene membrane without any sorbent present, derived during the K_{mw} laboratory experiments.

A fourth term was added to Equation 2 to account for the uptake resistance introduced by a bio fouled membrane. We estimated the thickness of the biological layer to be 0.04 cm based on observation, and used K_{oc} values reported by as a proxy for the affinity of PFAS to the organic material in the bio fouled layer:⁴²

$$\frac{1}{k_0} = \frac{1}{k_w} + \frac{1}{K_{mw}*(D_w*p_m/d_m)} + \frac{1}{K_{sw}*(D_w*p_s/d_s)} + \frac{1}{K_{oc}*(D_w*p_b/d_b)} \quad (\text{Equation 19})$$

The addition of the bio fouled layer as parameterized above resulted in a 0.2-2% decrease in the modeled sampling rate for PFHxA and PFHxS at 15 and 25 °C. These considerations, in concert with the laboratory results and the modeled simulation of the impact of an additional, thin layer of biofouling, indicate the impact on sampler uptake can be disregarded in the numerical model for predicting sampling rate of a field deployed passive sampler. Using the model, a 1 cm thick layer of biofouling was calculated to be the limit at which the sampling rate would decrease by more than 25% for PFHxA and PFHxS at flow speeds $>0.5 \text{ cm s}^{-1}$.

Uptake of PFAS in the field. PFHxA displayed sampling rates of $40 \pm 10 \text{ mL day}^{-1}$ in the riverine field deployments at high flows (Table S3). These values are not observably different given the overlapping uncertainties with the laboratory observed sampling rates ($100 \pm 80 \text{ mL}$

day⁻¹) and model predicted sampling rates (60 ± 30 mL day⁻¹). PFHxS sampling rates had slight differences between the field (50 ± 20 mL day⁻¹) and the more similar laboratory (100 ± 100 mL day⁻¹) and modeled (100 ± 40 mL day⁻¹) sampling rates. Tube passive samplers deployed in field conditions showed lower variance than the laboratory observed passive samplers with an average standard deviation of 43% in the field replicates compared to 61% in the laboratory. The good agreement between the field and laboratory observed sampling rates at similar conditions suggests that the tube sampler design is adequate for time weighted average measurements of PFAAs in the environment (**Table 28**).

Uptake of PFAS over time. Replicate passive samplers were deployed for 3, 7, 16, 28, and 54 days in the experimental tank to determine the length of time in which they will remain in linear uptake phase. The results show that linear uptake was maintained for the shorter chain PFCA and PFSA compounds, including PFHxA, PFHpA, PFBS, and PFHxS across 54 days (**Figure 34**). Compounds with higher K_{mw} generally showed little change in mass of PFAS between 16-54 days. These results suggest that a shorter deployment length of 14 days should be used to ensure all compounds are in the linear uptake phase during sampler deployment.

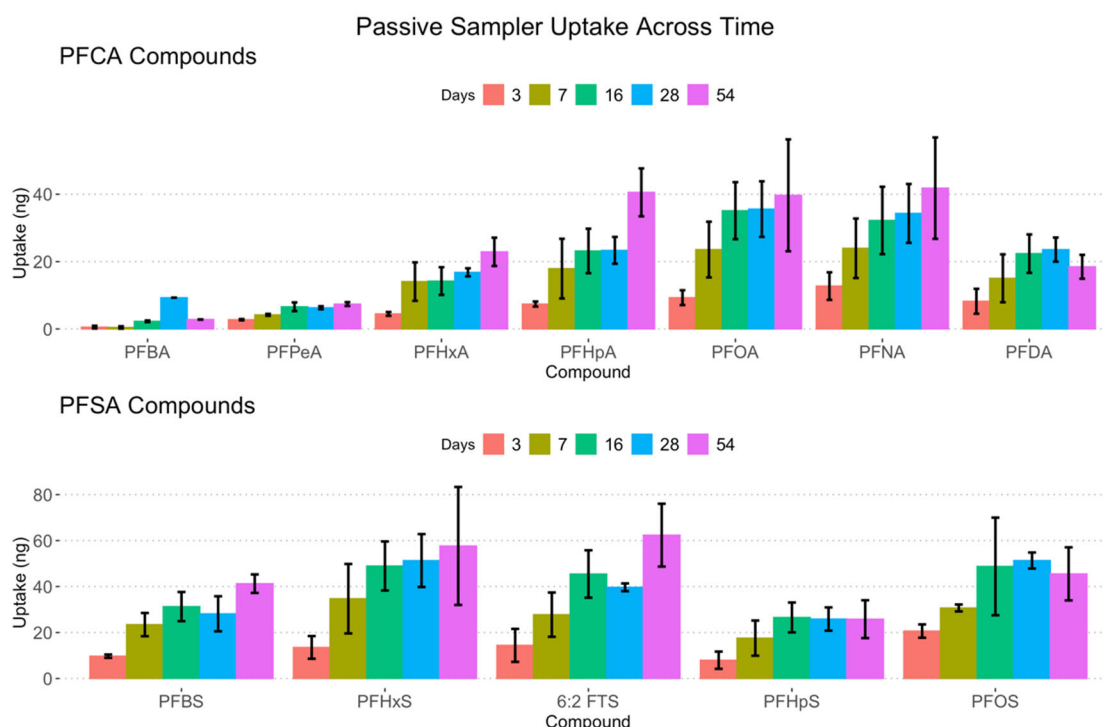


Figure 34. Mean uptake (ng) of PFAS by replicate passive samplers.

Error bars represent one standard deviation.

Comparison to other passive samplers for PFAA. The observed PFAS sampling rates were compared to published sampling rates from other integrative passive samplers, normalized to sampler surface area.^{20,23,24} The tube passive sampler compares favorably to the standard POCIS design, with a R_s/Area almost an order of magnitude higher (**Table 30**). It falls within the ranges

reported for DGT samplers, though it is lower than the reported modified POCIS method developed with a smaller surface area (**Table 30**)

Table 30. Comparison of PFHxA sampling rates normalized to surface area.

Sampler Design	Reported Rs (mL day ⁻¹)	Rs SD	Sampler Surface Area (cm ²)	Deployment Temp (°C)	Deployment Flow (cm s ⁻¹)	Rs/Area (cm day ⁻¹)
Modified POCIS ^a	290	0.05	16	27	23	18
POCIS ^b	29	.012	46	20	NR	0.63
Tube Passive Sampler (This Study)	48	.016	20.	25	20	2.3
DGT ^c	5.7e-6*	NR	0.064-0.21*	25	0	2.3-7.8*

^aData from Kaserzon et al. (2012). ^bData from Gobelius et al. (2019). ^cData from Fang et al. (2021). Because there are no reported sampling rates for PFHxA or PFHxS from the DGT sampler, an estimation of mass transfer (Rs/area) was derived from the reported diffusion coefficients of PFHxA through the agarose layer divided by the thickness of the agarose layer used to construct the sampler.

Comparison of 2- and 3-phase models. The two-phase model (Equation 5) routinely underestimated the observed Rs. The 2- and 3-phase model predictions became less similar (Equations 1 and 2) with an increase in PFAA chain length (**Table 28**). This two-phase model, that was derived for the thin membrane POCIS was not a sufficient choice for modeling tube passive sampler uptake when compared to the more thorough three phase model discussed in these results (**Table 28**) (Endo et al., 2019). The 2-phase model's lack of accounting for sorbent uptake or partitioning of PFAAs to sorbent and membrane.

Comparison of model, laboratory, and field sampling rates. Mean PFHxA sampling rates were 100 (±80) and 100 (±50) mL day⁻¹ in the laboratory experiments at 15 and 25 °C between 10-60 cm s⁻¹ flow speeds. At the same temperatures, the model predicted values of 60 (± 40) and 70 (±30) mL day⁻¹ which suggests these proposed values for PFHxA diffusivity, K_{mw}, and K_{sw} are relatively accurate. Field observed sampling rates, from 10 surface water deployed samplers were found to 40 (± 10) mL day⁻¹ for PFHxA at temperature approaching 15 °C. PFHxS displayed similarly promising results with observed sampling rates of 100 (± 100) and 200 (±70) mL day⁻¹ compared to predicted sampling rates of 100 (± 40) and 100 (±60) mL day⁻¹ for 15 and 25 °C respectively. Field observed sampling rates averaged out to 50 (± 20) mL day⁻¹ at temperatures near 15°C.

For 7 of the 9 PFAAs investigated in this study, the model predicted sampling rate and observed sampling rates were in good agreement, within a factor of 50% or less (**Table 28**). Standard deviation of replicates and uncertainty of the modeled sampling rates overlapped for PFPeA, PFHxA, PFHxS, PFHpA, PFHpS, PFOA, and PFOS (**Table 28**). There was a large

discrepancy between the observed sampling rates and modeled sampling rates by almost an order of magnitude for the shortest chain compounds PFBA and PFBS. Despite having generally lower K_{mw} than longer chain acids, these compounds had the highest aqueous diffusivities. The model could be prone to overestimate the sampling rate of these fast-diffusing compounds if the K_{mw} reported in **Figure 31** were anomalously high.

Environmental Implications. In this study, the passive sampler was shown to take up PFAA with 4-8 carbon chain lengths in a predictable manner in both the laboratory and field. The resistance to varying water flows exhibited by the tube passive sampler in this study (**Figure 28**, **Figure 29**) provides an advantage for widespread deployments in surface waters without the need for site specific calibration. The model can easily be tweaked for temperatures outside of the 15-25 °C discussed here, and a predicted sampling rate, or range of sampling rates, can be applied to riverine or estuarine flows $>0.5 \text{ cm s}^{-1}$. Further investigation of low flow conditions may be necessary for broad groundwater deployments, though the initial measured sampling rates from groundwater deployed samplers were in good agreement with the modeled predictions for 0.001 cm s^{-1} flow, which is the zero-condition used when samplers are deployed in stagnant water.

Future work should include an investigation of how an improved sorbent for PFAA uptake compared to HLB might increase sampler uptake over time. While the K_{mw} values for PFAA indicate that sorption to the membrane is a clear path of uptake. Deriving sampling rates for the membrane is possible and does not seem to outweigh the impact the sorbent plays on overall uptake kinetics (**Figure 1**). This suggests that the leveling off-of sampling uptake exhibited between 16-54 days could be due to sorbent congestion or inefficient transport of PFAS through the sorbent layer within the membrane as opposed to inhibition by a saturated membrane.

The novel tube passive sampler for PFAS is a flow-resistant solution to measuring time weighted averages of PFAS concentration in surface waters. A previously published numerical model for integrative passive sampler uptake can be used to predict sampling rate given a simple understanding of water temperature and a given PFAS compound's unique physico-chemical characteristics. The applications of this model and tube sampler can expand beyond legacy PFAA as a better understanding of novel PFAS diffusivities and other chemical properties are published. The tube passive sampler could be a useful tool for many stakeholders to broadly measure PFAS in the environment, leading to better decision making for risk assessment and policy decisions.

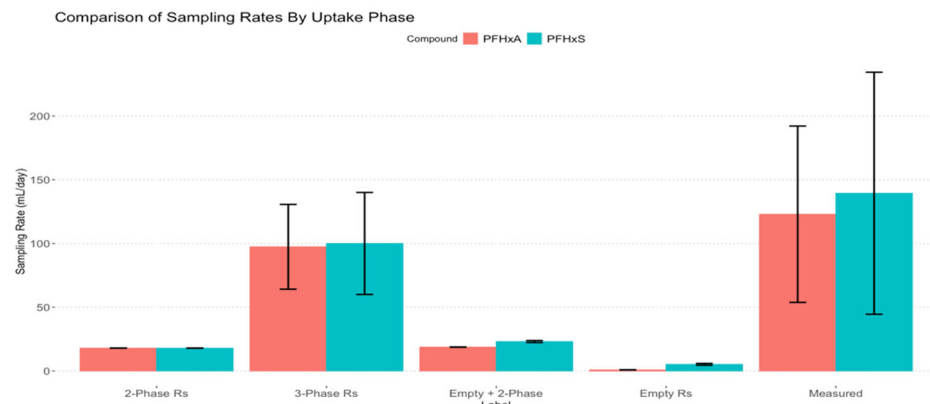


Figure 35. A comparison of sampling rates according to the number of phases of uptake.

Uncertainty of the sampling rate (mL/day) is overlaid as error bars. The “Empty” phase refers to the sampling rate of the polyethylene membrane without any sorbent present, derived during the K_{mw} laboratory experiments.

SUMMARY AND KEY FINDINGS

Results of this project help to better understand factors affecting the mobility and accumulation of PFAS in groundwater. We gained insights into how PFAA precursor transport and biotransformation are affected by varying geochemical conditions in groundwater environments at the JBCC site and surrounding areas that historically received PFAS inputs from AFFF use.

Implications, limitations of the research, and recommendations for future work

Task 1: Column Experiments. Previous work has shown predominant water chemistry factors that affect PFAS sorption include pH, dissolved organic carbon (DOC), salinity, and divalent cation concentrations (Higgins and Luthy, 2006; Du et al., 2014). The decline in sorption during the elution phase for PFAS with $K_d > 1$ (**Table 24**) was unlikely due pH and DOC because opposite trends from the literature were observed (Du et al., 2018). Changes in sorption were also unlikely due to conductivity because the salting out effect is not significant at the low conductivities ($< 125 \mu\text{S cm}^{-1}$) of the two groundwaters (You et al., 2010; Munoz et al., 2017). Therefore, the effect of divalent cations, which neutralizes, and bridges negatively charged surface sites and anionic PFAS, is the most plausible mechanism driving sorption in the experiment. A log-unit increase in the sum of calcium (Ca^{2+}) and magnesium (Mg^{2+}) associated with a 0.21 ± 0.06 log-unit increase in PFAS sorption. Observed changes in sorption of PFCA, PFSA, and FTSA were similar across functional groups, consistent with larger changes in Ca^{2+} (which can bridge all compounds due to its lower electronegativity) versus Mg^{2+} (which preferentially bridge carboxylates due to its higher electronegativity) between the two groundwaters (Wang et al., 2011; Kennedy et al., 2004). The role of divalent cation bridging which affects only deprotonated PFAS may also explain why no spike in concentration was observed for the FASA, which are expected to be only 3 – 27% anionic in the range of pH of the groundwaters (pK_a estimated by ARChem: FBSA = 6.59, FHxSA = 6.57) (ArChem, 2010).

Divalent cation bridging has significant field implications for PFCA retardation because large increases in divalent iron and manganese (which are both more electronegative than calcium) are observed in reducing regions of the PFAS plume at the field site (Weber et al., 2017). Divalent cation complexation of carboxylates is stronger than for sulfonates due to their lesser degree of hydration (Groenendijk et al., 2021). We hypothesize that reducing conditions observed in infiltrating groundwater from a downgradient lake could explain greater sorption of PFCA compared to PFSA in Tokranov et al. (2021). Future work should systematically explore the effect of sorbent electronegative (Ca^{2+} , Mg^{2+} , Fe^{2+} , and Mn^{2+}) and sorbate (PFCA vs. PFSA vs. FASA) on divalent cation bridging. These findings have general implications for AFFF contaminated sites and are not specific to JBCC.

Task 2: Microbial Degradation Experiments. The relative stability of precursors against oxidation into PFAA in soils allows for their transport through the vadose zone into groundwater and downstream watersheds (Tokranov et al., 2021). Environmental conditions and microbial communities vary along the flow path, especially near groundwater/surface water boundaries where large gradients in nutrients and redox conditions can be present (Barzen-Hanson et al., 2017, D’Agostino et al., 2017, Houtz et al., 2013, Ruyle et al., 2021b). Past work near the field sampling location for the groundwater/sediment slurries used in this study showed a distinct

seasonality in precursor transport across the groundwater/surface water boundary immediately downgradient from a legacy AFFF plume (Ashumet Pond, MA) (Tokranov et al., 2021). Rapid removal (<36 hours) of up to 85% of precursors was observed in field data during the summer months but not in the winter months.

These field-observations are consistent with the large and rapid (<1 day) bioassociation of precursors observed experimentally in this study (**Figure 16, Figure 17**). We estimated based on modeling that biotransformation of all precursors into PFHxS would require more than a year (**Figure 20**). These results suggest that some of the precursors and/or intermediate metabolites associated with the immobile microbiome in the field will be released back into the aqueous phase during winter months when turnover of microbial biomass occurs, which is consistent with prior field observations (Tokranov et al., 2021).

Experimental data from this study appear to show zero-order production of PFHxS (**Figure 18**). Such kinetics suggest microbial limitations on biotransformation rather than the availability of precursors. Theory based on Michaelis-Menten kinetics suggests that zero-order kinetics occurs when the substrate is much more abundant than the Michaelis constant (K_M) or the maximum rate of product formation is much less than the total time of the experiment (poor enzymatic efficiency). Typical values for K_M ($\geq 1 \mu\text{M}$) (Gatto et al., 2019) are orders of magnitude larger than the maximum precursor spike at the beginning of the experiments. This implies PFHxS production could only be enzyme-limited if sulfonamido precursors are in competition with other reduced nitrogen species for coordination sites. In the bioactive experiments, zero-order production of nitrate exceeded what could be potentially released upon complete biotransformation of the spiked precursor pool (**Figure 21**). Future work should identify specific enzymes responsible for biotransformation to understand the role of competing substrates and reaction efficiency on precursor reaction rates. These findings suggest a general relationship between nitrogen cycling and biotransformation of sulfonamido precursors at sites contaminated by 3M AFFF use that should be explored in future research and may be useful for site assessment.

Task 3: Model for precursor transport and transformation at JBCC former fire-training area.

We found that targeted terminal PFAS and the TOP+BI results could reproduce all the EOF in AFFF contaminated groundwater below the JBCC former fire-training area. This allowed us to quantitatively reconstruct the mass budget for PFAS in groundwater at JBCC and a similar technique could be employed at other sites. TOP+BI results indicated $80 \pm 5\%$ of the AFFF used at the JBCC former fire-training area were 3M products. Precursors accounted for approximately half ($46 \pm 8\%$) of the total PFAS in JBCC groundwater.

Concentrations of PFBS+C4 ECF precursors, PFHxS+C6 ECF precursors, PFOS+C8 ECF precursors, and PFOA showed large temporal fluctuations but no consistent trends. We hypothesized that dynamic vadose zone conditions (precipitation, evapotranspiration, and aquifer recharge) drive the variable PFAS concentrations measured in JBCC groundwater over time. We found statistically significant correlations ($p < 0.05$) between groundwater PFAS concentrations and the water altitude of the kettle lake for PFOA (C7 PFCA) and PFBS (C4 PFSA). Both PFOA and PFBS have relatively higher water solubilities than PFOS. PFOA also interacts strongly with the air-water interface so may be flushed through the vadose zone under saturated conditions. By

contrast, the higher sorption to solids and/or rate-limited partitioning for PFHxS, PFOS and precursors may dampen variability in aqueous transport for these compounds under dynamic soil moisture conditions. Future research should further explore drivers of temporal variability in groundwater PFAS concentrations in relationship to soil moisture, using direct measurements if possible.

The central estimates for the first-order precursor biotransformation rate from the data optimized model of the JBCC former fire-training area were $1 \times 10^{-3} \text{ y}^{-1}$ (C4 PFSA precursors) to $2 \times 10^{-3} \text{ y}^{-1}$ (C6 PFSA precursors) (IQR: $4 \times 10^{-4} - 1 \times 10^{-2} \text{ y}^{-1}$). Modeling results suggest that the C4 PFSA and C6 PFSA measured during sampling between 2007-2021 primarily reflect precursor biotransformation. In 2021, model results suggest 98% (interquartile range [IQR]: 92-99%) of the C4 PFSA and 56% of the C6 PFSA (IQR: 19-88%) in groundwater were formed through precursor biotransformation. Slow precursor biotransformation (half-lives = 340-670 yr; minimum = 66 yr), indicates that some precursors persist and are transported to downstream environments where potential deleterious exposures occur. Conversely, precursor biotransformation appears to have a minimal impact on the C8 PFSA reservoir due to low relative abundance of C8 PFSA precursors in 3M AFFF. This result suggests precursor biotransformation is likely to be a dominant contributor to groundwater PFBS and PFHxS contamination at legacy AFFF-contaminated sites. Future research should extend the findings and modeling from JBCC to examine the importance of these processes. Results suggest reservoirs of precursors in the vadose zone need to be addressed during site management, not just the terminal PFAA in 3M AFFF.

Task 4: Passive Samplers. In this study, the passive sampler was shown to take up PFAA with 4-8 carbon chain lengths in a predictable manner in both the laboratory and field. The resistance to varying water flows exhibited by the tube passive sampler in this study (**Figure 28**, **Figure 29**) provides an advantage for widespread deployments in surface waters without the need for site specific calibration. The model can easily be tweaked for temperatures outside of the 15-25 °C discussed here, and a predicted sampling rate, or range of sampling rates, can be applied to riverine or estuarine flows $>0.5 \text{ cm s}^{-1}$. Further investigation of low flow conditions may be necessary for broad groundwater deployments, though the initial measured sampling rates from groundwater deployed samplers were in good agreement with the modeled predictions for 0.001 cm s^{-1} flow, which is the zero-condition used when samplers are deployed in stagnant water.

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The novel tube passive sampler for PFAS is a flow-resistant solution to measuring time weighted averages of PFAS concentration in surface waters. A previously published numerical

model for integrative passive sampler uptake can be used to predict sampling rate given a simple understanding of water temperature and a given PFAS compound's unique physico-chemical characteristics. The applications of this model and tube sampler can expand beyond legacy PFAA as a better understanding of novel PFAS diffusivities and other chemical properties are published. The tube passive sampler could be a useful tool for many stakeholders to broadly measure PFAS in the environment, leading to better decision making for risk assessment and policy decisions.

Key findings from Task 1.

- Lab-derived K_d values agreed with field-derived K_d within the field-derived uncertainty bounds previously reported in Weber et al. (2017) (maximum of an order of magnitude).
- Trends in site-specific sorption at JBCC depended on the functional group and followed the order PFCA < PFSA < FTSA < FASA.
- Results of column experiments suggest divalent cation bridging is the main geochemical mechanism controlling sorption at the site, particularly for PFCA.
- Divalent cation bridging has significant field implications for PFCA retardation because large increases in divalent iron and manganese (which are both more electronegative than calcium) are observed in reducing regions.
- Future work should systematically explore the effect of sorbent electronegative (Ca^{2+} , Mg^{2+} , Fe^{2+} , and Mn^{2+}) and sorbate (PFCA vs. PFSA vs. FASA) on divalent cation bridging.

Key findings from Task 2.

- For the three C6 sulfonamido precursors included in this study (PFHxSA_m, PFHxSA_mS, FHxSA), the rate constants for sorption/association with microbes ranged between -0.2 to -5.0 days⁻¹.
- At 29°C, the first order half-life of PFHxSA_mS and PFHxSA_m was less than half a day and approximately 5x faster than the bioassociation of FHxSA.
- Biotransformation of all three precursors was indicated by formation of PFHxS, the terminal product of microbial biotransformation of C6 sulfonamido precursors.
- Formation of PFHxS appeared to proceed at a constant rate throughout the experiment regardless of any changes in the concentrations of reactants (precursors), implying that biotransformation was limited by the microbial reactions catalyzing this transformation.
- Despite faster microbial association of the zwitterions compared to FHxSA, the rate limiting step for PFHxS production was the oxidation of the zwitterions to FHxSA rather

than oxidation of FHxSA to PFHxS, explaining minimal accumulation of FHxSA observed in the bioactive experiments.

- These findings suggest a general relationship between nitrogen cycling and biotransformation of sulfonamido precursors at sites contaminated by 3M AFFF use that should be explored in future research.

Key findings from Task 3

- In groundwater below the JBCC fire training area, large temporal variability in PFAS concentrations but no clear temporal patterns were observed.
- Temporal fluctuations for terminal PFAS with fewer precursors (PFOA) and the most hydrophilic PFAS (PFBS) were significantly ($p < 0.05$) correlated with soil moisture, indicating saturation effectively flushes these PFAS through the vadose zone into groundwater.
- No correlation ($p > 0.05$) was observed for other PFAS, including all precursor classes, that have greater propensity for sorption and high surface-activity (high K_d and K_{ai}).
- Numerical modeling constrained by measurements suggested that soil contains a much larger reservoir of PFAS than groundwater.
- Biotransformation of precursors is a substantial contributor to ongoing contamination.

Key findings from Task 4

- A microporous polyethylene tube filled with a hydrophilic-lipophilic balance (HLB) sorbent effectively serves as a flow-resistant passive sampler for PFAS.
- Good qualitative agreement among lab tank deployments, field deployments and numerical modeling was observed for passive sampler.
- Biofouling did not substantially affect uptake by the passive sampler.

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APPENDIX A. INTERLABORATORY COMPARISON

The PFAS Analytical Confirmation (Summary in **Table 31**) was completed by comparing three laboratories; Sunderland Lab at Harvard University, Lohmann Lab at University of Rhode Island, and the DoD ELAP-Accredited Gel Labs. Six water samples were collected by Harvard University and split samples were shipped on ice to URI and Gel Labs. The three individual labs then completed sample preparation, extraction, and LC-MS/MS analysis. The quantitative LC-MS/MS PFAS data from all three labs was compiled by Harvard University and analyzed.

Water samples were collected at six different locations on Cape Cod using pre-cleaned HDPE bottles. Samples and duplicates were collected by submersing the bottle downstream of the water flow, rinsing the bottle 3x with sample, and filling the bottle and capping tightly. Field blanks were collected by pouring Milli-Q water from one bottle into an empty pre-cleaned field blank bottle. Samples were packaged into plastic bags and put in coolers on ice for transportation back to the lab where they were either shipped on ice to the other labs or kept in a refrigerator (2 oC) until preparation, extraction, and LC-MS/MS analysis.

A total of thirty-five PFAS were analyzed in six water samples but only twenty-four of these PFAS were analyzed at all three labs, giving a total of 144 data points for these twenty-four compounds and 210 data points for the thirty-five compounds. The data from the three labs was compared by calculating the coefficient of variation (CV) for all 210 data points. The CV for 98 of the points was N/A since two out of the three labs received a non-detect (ND), was lower than method detection limits (<MDL) or was not analyzed for and thus a CV could not be calculated. An attempt was made to estimate the PFAS concentration from the known MDL using the $\sqrt{2}/2$ method; however, the MDL values for the three labs typically ranged by factors of 4-40 yielding unreasonable estimations. Thus these 98 N/A points were not utilized for further comparison.

For the remaining 112 points, the CV range was 0.8 to 85%, the mean was 26%, the median was 21%, 80 points (71%) had a CV <30%, and 32 points (29%) had a CV >30%. For the 32 with a CV >30%, 20 PFAS data points had a ND or <MDL from one of the three labs explaining these results. If these 20 points are removed, there is a reduction in CV range to 0.8-46%, mean to 21% and median to 19%. Of the remaining data points, the CV is only high (>30%) for compounds with levels just above the MDL.

Relative percent differences (RPD) were calculated between each pair of labs for every sample and PFAS analyte when concentrations >MDL were determined by both labs. RPD between the Sunderland Lab and Gel Lab ranged from 1 to 91% with a mean of 35%, median of 36%, and 40% of the data had RPD $\leq$ 30%. RPD between the Lohmann Lab and Gel Lab ranged from 0 to 94% with a mean of 30%, median of 23%, and 65% of the data had RPD $\leq$ 30%. RPD between the Lohmann Lab and Sunderland Lab ranged from 1 to 169% with a mean of 49%, median of 39%, and 29% of the data had RPD $\leq$ 30%.

Method detection limits (MDL) were determined as the instrument limits of detection (LOD) multiplied by each sample's dilution volume. LOD were calculated as the concentration at which the average sample signal-to-noise ratio is 3. The Sunderland Lab specifically calculates

there LOD using only samples above the lowest calibration point and below the highest calibration point and determine a sample LOD by taking the average of the LOD calculated for each sample and multiplying by 3. Instrument limits of quantification (LOQ) were calculated for a signal-to-noise ratio of 10 and therefore method quantification limits (MQL) were determined as the LOQ multiplied by the sample dilution volume.

For QC summary, the Sunderland Lab at Harvard included eight instrument blanks, one method blank, two field blanks, one sample spike, and one sample duplicate in their analysis of twenty-eight compounds (including branched). All instrument blanks had concentrations <MDL. All method/field blanks had concentrations <MDL, except for one compound, PFOA, which had concentrations just above MDL, but <MQL. The mean sample spike recovery for all twenty-eight compounds was 108% (median: 109%, range: 72-135%). Only PFTrDA (135%) was just outside the range of 70-130%. The sample duplicate average RPD for all compounds was 4.35% (median: 3.96%, range: 0-9.52%). All compounds had an RPD < 10%. The average internal standard (IS) recoveries for the blanks and samples versus the calibration were calculated with a mean recovery of 85% and 93%, respectively. Average IS recovery in the blanks/calibration (median: 84%, range: 77-91%) and samples/calibration (median: 80%, range: 51-272%) were within the 70-130% range for all compounds, except the shortest-chain PFAS; PFBA, 4:2 FtS, and 6:2 FtS in the samples, due to IS interference with sample matrix.

For QC summary, the Lohmann Lab at URI included two process blanks and four field blanks. In all blanks, the analyzed compounds were below limits of detection. Additionally, two laboratory control samples (each run with one sample batch) as well as two matrix spiked (in duplicate) were included. The overall recoveries for all spiked samples varied between 72 and 110%. The lower recoveries for PFHxDA (60%) when spiking 10 ng/sample were caused by PFHxDA surface sorption in combination with M2PFTeDA used as an indirect internal standard. The internal standard (IS) recoveries were calculated as a ratio between IS area in every sample and average IS area in all calibration solutions. Internal standard recoveries in all blanks varied between 65 and 126% (average 92%) and 63 and 116% (average 83%) for all analyzed samples.

Table 31. Intercomparison of PFAS concentrations (ng/L) in water samples determined by Sunderland, Lohmann, and Gel Labs.

Sample	Lab	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnDA	PFDoDA	PFTrDA	PFTeDA	FOSA	MeFOSA	EtFOSA	N-MeFOSAA	N-EtFOSAA
QR09	GEL Lab	4.01	12.4	15.5	8.11	18	9.57	<0.688	<0.582	<0.582	<0.582	<0.582	<1.16	<1.16	<0.582	<1.16	<1.16
	Lohmann Lab	4.80	10.08	13.2	5.55	11.28	7.02	0.15	<0.09	<0.11	<0.18	<0.15	0.81	NA	NA	QL	<0.06
	Sunderland Lab	3.50	8.32	11.57	5.22	11.42	6.00	0.06	QL	<0.02	<0.03	<0.04	0.25	NA	NA	0.06	0.10
MCO1	GEL Lab	15.5	50.2	30.9	15.5	12.3	5.06	<0.707	<0.598	<0.598	<0.598	<0.598	<1.20	<0.598	<1.20	<1.20	<1.20
	Lohmann Lab	11.8	50.87	30.6	12.4	9.65	4.36	0.46	<0.09	<0.11	<0.18	<0.15	0.88	NA	NA	0.20	0.21
	Sunderland Lab	8.29	34.32	23.49	9.72	8.52	3.91	0.41	0.11	0.10	0.11	0.09	0.53	NA	NA	QH	0.12
MDPWC	GEL Lab	3.6	6.32	7.23	6.02	2.76	2.95	<0.703	<0.595	<0.595	<0.595	<0.595	<1.19	<0.595	<1.19	<1.19	<1.19
	Lohmann Lab	2.86	5.09	8.51	6.17	3.32	2.69	0.65	<0.09	<0.11	<0.18	<0.15	0.26	NA	NA	QL	<0.06
	Sunderland Lab	3.26	4.85	5.22	4.31	1.93	2.19	0.08	0.04	<0.02	<0.03	<0.04	<0.08	NA	NA	<0.03	<0.07
FPBC	GEL Lab	20.2	55.1	75.9	54.5	35.2	49.7	8.36	10.5	<0.593	0.684	<0.593	<0.593	<1.19	<0.593	<1.19	<1.19
	Lohmann Lab	22.4	53.2	65.4	43.6	27.8	51.7	6.67	7.32	<0.11	<0.18	0.22	0.51	NA	NA	0.16	0.19
	Sunderland Lab	14.04	43.49	48.87	36.35	24.46	32.12	4.32	5.35	QH	QH	0.09	0.14	NA	NA	<0.03	<0.07
MAPAP	GEL Lab	1.27	1.5	1.36	0.82	1.37	<0.598	<0.707	<0.598	<0.598	<0.598	<0.598	<1.20	<0.598	<1.20	<1.20	<1.20
	Lohmann Lab	2.44	<0.16	1.14	0.4	1.50	0.42	0.32	<0.09	<0.11	<0.18	<0.15	<0.11	NA	NA	0.47	<0.06
	Sunderland Lab	1.32	0.71	1.01	0.57	0.87	0.23	0.07	0.04	<0.02	<0.03	<0.04	<0.08	NA	NA	0.04	<0.07
MAR3	GEL Lab	0.894	1.31	1.61	0.651	1.8	<0.587	<0.694	<0.587	<0.587	<0.587	<0.587	<1.17	<0.587	<1.17	<1.17	<1.17
	Lohmann Lab	2.48	<0.16	0.97	0.29	2.38	0.25	0.27	<0.09	<0.11	<0.18	0.33	0.40	NA	NA	QL	<0.06
	Sunderland Lab	<1.14	0.72	1.05	0.48	1.15	0.17	0.07	<0.03	<0.02	<0.03	<0.04	<0.08	NA	NA	<0.03	0.08

Sample	Lab	PFBS	PFPeS	PFHxS	PFHpS	PFOA	PFNA	PFDA	PFUnDA	PFDoDA	PFTrDA	PFTeDA	FOSA	MeFOSA	EtFOSA	N-MeFOSAA	N-EtFOSAA
QR09	GEL Lab	4.16	4.27	NA	NA	53.4	1.14	NA	NA	72.8	<0.618	<0.582	<1.16	7.91	<1.16	NA	NA
	Lohmann Lab	4.03	2.93	45.8	7.47	53.2	0.91	49.8	26.6	76.4	<0.09	<0.14	QL	3.41	0.88	1.12	4.14
	Sunderland Lab	3.33	3.57	31.78	5.46	37.24	1.35	31.14	14.42	45.56	<0.07	<0.03	QL	5.61	0.99	0.77	4.55
MCO1	GEL Lab	3.86	1.91	NA	NA	22.8	0.858	NA	NA	41.4	<0.634	<0.598	<1.20	56.5	<1.20	NA	NA
	Lohmann Lab	2.93	1.10	20.6	2.78	23.4	0.64	31.3	14.6	46.0	<0.09	<0.14	QL	55.8	0.34	0.83	0.54
	Sunderland Lab	3.06	1.72	14.56	2.22	16.78	1.20	20.50	10.62	31.12	0.08	0.11	QL	36.89	0.11	0.46	0.63
MDPWC	GEL Lab	1.52	2.59	NA	NA	18.3	<0.595	NA	NA	22.7	<0.631	<0.595	<1.19	<1.19	<1.19	NA	NA
	Lohmann Lab	2.81	1.13	13.5	1.91	15.4	0.21	17.0	7.15	24.1	<0.09	<0.14	0.23	0.61	<0.28	0.32	<0.06
	Sunderland Lab	1.53	1.54	9.23	1.34	10.57	0.51	11.27	5.55	16.82	<0.07	<0.03	0.06	<0.06	<0.06	0.16	<0.09
FPBC	GEL Lab	6.05	10.1	NA	NA	86	5.63	NA	NA	382	<0.629	<0.593	<1.19	6.06	<1.19	NA	NA
	Lohmann Lab	4.18	4.37	75.5	11.8	87.3	4.90	240	64.9	305	1.25	1.44	0.29	6.20	QL	3.14	5.76
	Sunderland Lab	4.95	6.77	52.30	7.07	59.37	8.06	202.46	63.90	266.36	0.29	0.12	0.06	4.37	1.04	2.28	8.95
MAPAP	GEL Lab	0.8	<0.598	NA	NA	0.793	<0.598	NA	NA	0.882	<0.635	<0.598	<1.20	<1.20	<1.20	NA	NA
	Lohmann Lab	1.80	QL	1.42	QL	1.42	<0.11	0.86	0.39	1.25	<0.09	<0.14	<0.14	<0.11	<0.28	<0.03	<0.06
	Sunderland Lab	0.86	<0.26	0.49	QL	0.49	<0.20	0.33	QL	0.33	<0.07	<0.03	QL	<0.06	<0.06	0.14	<0.09
MAR3	GEL Lab	1.23	<0.587	NA	NA	0.799	<0.587	NA	NA	1.07	<0.623	<0.587	<1.17	<1.17	<1.17	NA	NA
	Lohmann Lab	2.11	0.11	1.40	0.27	1.67	<0.11	1.41	0.44	1.86	<0.09	<0.14	QL	1.7	0.5	<0.03	<0.06
	Sunderland Lab	1.19	<0.26	0.51	0.13	0.64	<0.20	0.41	0.41	0.82	<0.07	<0.03	QL	<0.06	<0.06	0.09	<0.09

PFAS below detection in samples are indicated by <MDL value, NA = not analyzed by the lab, QL = qualifier peak flagged low, QH = qualifier peak flagged high.

APPENDIX B: LIST OF PUBLICATIONS

- DeSilva, A.O.; Armitage, J.M.; Bruton, T.A.; Dassuncao, C.; Heiger-Bernays, W.; Hu, X.C.; Karman, A.; Ng, C.; Robuck, A.; Sun, M.; Webster, T.F.; Sunderland, E.M. PFAS Exposure Pathways for Humans and Wildlife: A Synthesis of Current Knowledge and Key Gaps in Understanding. *Environ. Toxicol. Chem.*, 40(3): 631-657.
- Ruyle, B. J.; Pickard, H. M.; LeBlanc, D. R.; Tokranov, A. K.; Thackray, C. P.; Hu, X. C.; Vecitis, C. D.; Sunderland, E. M. Isolating the AFFF Signature in Coastal Watersheds Using Oxidizable PFAS Precursors and Unexplained Organofluorine. *Environ. Sci. Technol.* 2021b, 55 (6), 3686–3695. <https://doi.org/10.1021/acs.est.0c07296>.
- Ruyle, B. J.; Thackray, C. P.; McCord, J. P.; Strynar, M. J.; Mauge-Lewis, K. A.; Fenton, S. E.; Sunderland, E. M. Reconstructing the Composition of Per- and Polyfluoroalkyl Substances in Contemporary Aqueous Film-Forming Foams. *Environ. Sci. Technol. Lett.* 2021a, 8 (1), 59–65. <https://doi.org/10.1021/acs.estlett.0c00798>.
- Ng, C., Cousins, I.T.; Glüge, J. ; Goldenman, G.; Herzke, D.; Lohmann, R.; Patton, S.; Scheringer, M.; Trier, X.; Wang, Z. Urgent Questions for PFAS in the 21st Century. *Environ Sci. Technol.* 2021, 55, 12755–12765. <https://doi.org/10.1021/acs.est.1c03386>
- Glüge, J.; London, R.; Cousins, I.T.; DeWit, J.; Goldenman, G.; Herzke, D.; Lohmann, R.; Miller, M.; Ng, C.A.; Patton, S.; Trier, X.; Wang, Z.; Scheringer, M. Information Requirements under the Essential-use Concept: PFAS Case Studies. *Environ. Sci. Technol.* 2021, *accepted*. <https://doi.org/10.1021/acs.est.1c03732>
- Manuscripts in Preparation/Review:
- Dunn, M.; Becanova, J.; Snook, J.; Ruyle, B.; Lohmann, R. Validation of Novel Tube Passive Samper for Perfluorinated Alkyl Acids.
- Ruyle, B.J.; Thackray, C.P.; LeBlanc, D.R.; Tokranov, A.K.; Vecitis, C.D.; Sunderland, E.M. Understanding the Fate of PFAS precursors at AFFF Contaminated Sites.
- Ruyle B.J., Shultes S., Akob D.M., Harris C.R., Lorah M.M., Vojta S., Becanova J., McCann S., Pickard H.M., Pearson A., Lohmann R., Vecitis C.D., Sunderland E.M. Nitrifying Bacteria Linked to Biotransformation of Perfluoroalkyl Sulfonamido Precursors from Legacy Aqueous Film Forming Foam.
- Gardiner, C.L.; Becanova, J.; Cantwell, M.G.; Katz, D.R.; Robuck A.R.; Lohmann, R. Evaluation of a Novel Passive Sampler for Poly- and Perfluorinated Alkyl Substances in Aquatic Environments.